(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
7 September 2001 (07.09.2001)

PCT

(10) International Publication Number
WO 01/64749 A2

(51) International Patent Classification[7]: C07K 16/00

(21) International Application Number: PCT/US01/05933

(22) International Filing Date: 26 February 2001 (26.02.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/185,390	28 February 2000 (28.02.2000)	US
60/233,625	18 September 2000 (18.09.2000)	US

(71) Applicant: **IDEC PHARMACEUTICALS CORPORATION** [US/US]; 11011 Torreyana Road, San Diego, CA 92121 (US).

(72) Inventors: **KLOETZER, William, S.**; 4317 Sea Bright Drive, Carlsbad, CA 92008 (US). **HANNA, Nabil**; 14770 Avenida Insurgentes, Rancho Santa Fe, CA 92067 (US).

(74) Agents: **TESKIN, Robin, L.** et al.; Pillsbury Winthrop LLP, 1100 New York Avenue, N.W., Washington, DC 20005 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/64749 A2

(54) Title: METHOD FOR PREPARING ANTI-MIF ANTIBODIES

(57) Abstract: The specification provides methods of preparing high-affinity antibodies to a macrophage migration inhibitory factor (MIF) in animals in which the *MIF* gene has been homozygously knocked-out ($MIF^{-/-}$). Also provided are methods of preparing hybridomas which produce the anti-MIF antibodies, methods of administering the antibodies to treat inflammatory or cancerous conditions and/or diseases modulated by MIF, as well as compositions comprising said high-affinity anti-MIF antibodies.

METHOD FOR PREPARING ANTI-MIF ANTIBODIES

Field of the Invention

The invention relates to a method of making high-affinity anti-macrophage migration inhibitory factor (MIF) antibodies in animals which are homozygously deficient of a *MIF* gene *(MIF$^{-/-}$)*. The invention further relates to high affinity anti-MIF antibodies, compositions comprising said antibodies and methods of treating diseases using said anti-MIF antibodies.

Background of the Invention

Migration Inhibitory Factor

Macrophage migration inhibitor factor (MIF) was one of the first identified lymphokines [Bloom *et al., Science* 153: 80-82 (1966)] and is a pleiotropic cytokine released by macrophages, T-cells and the pituitary gland during inflammatory responses. It acts as a pro-inflammatory cytokine, playing a major role in endotoxin shock and counter-regulating the anti-inflammatory effects of dexamethasone [Bozza *et al., J. Exp. Med.* 189: 341-6 (1999)]. MIF promotes tumor necrosis factor alpha (TNF∀) synthesis, T-cell activation [Leech *et al., Arthritis Rheum.* 42: 1601-8 (1999)], enhances interleukin-1 (IL-1) and interferon gamma (IFN() production [Todo, *Mol. Med.* 4: 707-14 (1998)], impacts macrophage-macrophage adherence, up-regulates HLA-DR, increases nitric oxide synthase and nitric oxide concentrations, and inhibits *Mycoplasma avium* growth (U.S. Patent No. 5,681,724). Certain of these features indicate that MIF also plays a role in the pathogenesis of rheumatoid arthritis (RA) (I*d.*). MIF is implicated in the activation of macrophages and counter-regulation of glucocorticoid activity [Chesney *et al., Mol. Med.* 5: 181-91 (1999)]. Recombinant forms of MIF and the DNAs encoding them have been previously described, see for example (WO 90/11301). MIF also has a reported role in the innate host response to staphylococcal and streptococcal exotoxins (Calandra *et al., Proc. Natl. Acad. Sci. USA* 95: 11383-8 (1998)).

MIF inhibition has been suggested for the treatment of acute lung injury to suppress the level of neutrophil attraction to the site of injury (Makita *et al., Am J.*

Respir. Crit. Care Med. 158: 573-9 (1998)). MIF localizes to the cytoplasm of leukemic cells and has been linked to a role in leukemia associated inflammatory events (Nishihira *et al., Biochem. Mol. Biol. Int.* 40: 861-9 (1996)).

Several forms of MIF have been identified. The first characterized was that of Weiser *et al., Proc. Natl. Acad. Sci. USA* 86: 7522-6 (1989). This MIF (MIF-1) is 115 amino acids and 12.5 kDa *(Id.)*. MIF-2 is a 45 kD protein identified in a human T-cell hybridoma clone (F5) (Hirose *et al., Microbiol. Immunol.* 35: 235-45 (1991)). The sequence of MIF-2 is very similar to MIF-l, but differs in that it is a more hydrophilic species than MIF-2 (Oki *et al., Lymphokine Cytokine Res.* 10: 273-80 (1991)).

MIF-3 is an 119 amino acid residue sequence (ATCC No. 75712; WO 95/31468). Antibodies and antagonists have been developed to MIF-3, which can be used to protect against lethal endotoxemia and septic shock or to treat ocular inflammations (WO 95/31468).

A related protein to MIF is the glycosylation-inhibiting factor (GIF), (Galat *et al., Eur. J Biochem.* 224: 417-21 (1994)). The cDNA expressing the human form of GIF is described by Mikayama *et al., Proc. Natl. Acad. Sci. U.S.A.* 90: 10056-60 (1993). The amino acid sequences for MIF-1 and GIF are now recognized to be identical. The correct amino acid sequence is 114 amino acids and forms a 12,345 Da protein (Swiss-Protein accession number P14174).

Anti-MIF Antibodies

Polyclonal and monoclonal anti-human MIF antibodies have been developed against recombinant human MIF (Shimizu *et al., FEBS Lett* 381: 199-202 (1996); Japanese Patent No. 9077799A; German Democratic Republic Patent No. 230876A; European Patent No. 162812; and ATCC Accession Nos. 00201X0003, 1024674 and 1014477). One monoclonal antibody against human MIF (IC5/B) has been developed and utilized to study signals to mononuclear phagocytes in pseudolymphomas and sarcoidosis [Gomez *et al., Arch. Dermatol. Res. (Germany)* 282: 374-8 (1990); see also Weiser *et al., Cell. Immunol. 90:* 167-78 (1985)]. Additional human monoclonal anti-MIF antibodies were developed by Kawaguchi *et al., J. Leukoc. Biol.* 39: 223-232 (1986) and Weiser *et al., Cell. Immunol.* 90: 167-78 (1985). Anti-murine MIF

monoclonal antibodies have also been prepared [See, *e.g.,* Malorny *et al., Clin. Exp. Immunol.* 71: 164-70 (1988); and Liu *et al., J. Immunol.* 137: 448-55 (1986)].

Anti-MIF antibodies have been suggested for therapeutic use to inhibit TNF∀ release (Leech *et al.,* 1999). As such, anti-MIF antibodies may have wide therapeutic applications for the treatment of inflammatory diseases. Related thereto, the administration of anti-MIF antibodies also reportedly inhibited adjuvant arthritis in rats (Leech *et al., Arthritis Rheum.* 41: 910-7 (1998)).

MIF has also been implicated in the pathogenesis of immunologically induced kidney disease. Lan *et al., J. Exp. Med.* 185: 1455-65 (1997) proposed the use of agents which block MIF activity to treat rapidly progressive glomerulonephritis in patients, and also suggested that MIF may be important in immune-mediated diseases generally.

Calandra *et al., I. Inflamm.* 47: 39-51 (1995) reportedly used anti-MIF antibodies to protect animals from experimentally induced gram-negative and gram-positive septic shock. Anti-MIF antibodies were suggested as a means of therapy to modulate cytokine production in septic shock and other inflammatory disease states *(Id.).*

Anti-MIF antibodies have been proposed for use to treat diseases where cellular/mucosal immunity should be stimulated or as a diagnostic or prognostic marker in pathological conditions involving the production of MIF (WO 96/09389).

MIF antagonists have been proposed to treat lymphomas and solid tumors which require neovascularization, (WO 98/17314). WO 98/17314 by Bucala *et al.* reportedly describes inhibition of murine Bull lymphoma growth *in vivo* by a neutralizing monoclonal antibody against MIF administered at the time of tumor implantation (Chesney *et al.,* 1999). Previous studies have shown that TH2 lymphacytes produce higher amounts of MIF upon stimulation than TH1 cells. (Bacher *et al,* 1996. PNAS 93:7849.) Since MIF is functionally involved in T-cell activation, neutralization of TH2 cell-derived may promote the ratio of TH1 to TH2 cells, thereby also prevent influencing host immunity against tumors (Chesney, 1999). Also, the use of anti-MIF antibodies for inhibiting proliferation of human endothelial cells has been reported [Chesney *et al., Mol. Med.* 5: 181-91 (1999); and Ogawa *et al., Cytokine* 12:309-314 (2000)]. Specifically, Ogawa et al. (2000) showed that

certain anti-MIF antibodies directly block VEGF stimulated endothelial cell growth, presumably through neutralization of endogenously produced MIF.

Knock-Out Animals for Use in Preparing Antibodies to Self-Antigens

Transgenic animals have been prepared wherein foreign antigens are now expressed in the transgenic animal as a self-antigen. For example, a virus protein was expressed in a transgenic mouse model as a self-antigen in the pancreatic islets of Langerhans, as described by Oldstone *et al., Cell* 65: 319-31 (1991). Typically, however, it is difficult to produce antibodies against self-antigens or autoantigens such as MIF. Autoantigens are normal constituents of the body, which remain typically are not recognized by the immune system.

A knock-out (KO) mouse or animal is one in which the animal is homozygously deficient of a functional gene (Declerck *et al., J. Biol. Chem.* 270: 8397-8400 (1995)). In general, antibodies will not be raised against self-antigens nor against highly conserved domains of proteins that do not vary between species. However, certain KO mice have been produced in which monoclonal auto-antibodies against various autoantigens have been raised. Castrop *et al., Immunobiol.* 193: 281-7 (1995) reported preparation of the use of a KO mouse for the generation of monoclonal antibodies to T-cell factor-1 (TCF-l), which had been historically difficult to prepare antibodies to due to the extreme evolutionary conservation of TCF- 1. Reportedly, because TCF- 1 is highly expressed in thymus, intrathymic selection mechanisms will impose tolerance for TCF- 1 in the immune system, likely through clonal deletion of TCF- 1-reactive T-cells *(Id.)*. The anti-TCF-l antibodies were raised against a fusion protein comprising TCF-1 fused to maltose binding protein (MBP).

LaTemple *et al., Xenotransplantation* 5: 191-6 (1998) used a KO mouse to ∀1,3galactosyltransferase (∀l,3GT KO) to produce a natural, anti-Gal antibody. However, the antibody was only produced in low amounts.

Declerck *et al.,* (1995) reported the preparation of anti-murine tissue-type plasminogen activator (t-PA) in a KO mouse, wherein the mouse lacked a functional t-PA gene. Declerck *et al.,* also suggested that this approach could be applied to other classes of proteins allowing the generation of monoclonal antibodies against conserved epitopes, which could not be raised in wild-type animals because of their

"self-antigen" nature. See also Declerck *et al.*, *Thromb. Haemost (Germany)* 74: 1305-9 (1995).

To better study the biologic role of MIF, a mouse strain lacking MIF was generated by gene targeting in embryonic stem cells (Bozza *et al.*, 1999). Using this mouse model, Bozza *et al.* determined that MIF is involved in a host response to gram negative bacteria induced sepsis.

Therefore, not withstanding what has been previously reported in the literature, there exists a need for preparing anti-MIF antibodies, especially monoclonal antibodies and fragments thereof with improved affinity and avidity for purposes of studying MIF function as well as regulating MIF activity. The methods of preparing the antibodies of this invention, as well as the antibodies themselves, can in turn be used to modulate MIF activity in diseases and conditions mediated by MIF, such as sepsis, rheumatoid arthritis, other autoimmune diseases, cancer, as well as injuries which induce MIF production.

Summary of the Invention

It is an object of the invention to provide novel and improved methods for producing high-affinity anti-MIF antibodies in animals which are homozygous deficient for a *MIF* gene *($MIF^{-/-}$)*. The gene can be *MIF-1, MIF-2, MIF-3* or a MIF-like gene, but preferably is the MIF described by Weiser *et al.*, (1989). A preferred method for preparing high affinity anti-MIF antibody or immunogenic fragment thereof comprises the steps of: (A) preparing a transgenic animal in which the *MIF* gene is functionally knocked out; (B) immunizing said transgenic animal with MIF or an immunogenic polypeptide fragment thereof; and (C) obtaining anti-MIF antibodies from said animal.

It is a more specific object of the invention to provide a novel method of preparing high-affinity anti-MIF antibody fragments, such as Fv, Fab, $F(ab')_2$, Fab' and scFV.

Another object of the invention is to provide for a method of obtaining cells which produce high-affinity anti-MIF antibodies from a MIF knock-out animal for purposes of preparing anti-MIF antibody producing cell lines or cell lines which produce recombinant forms of anti-MIF antibody fragments.

Another object of the invention is to provide a novel nucleic acid encoding a *MIF* gene targeting construct comprising (A) a selectable marker and (B) DNA sequence homologous to a *MIF* gene or portion thereof, wherein said isolated nucleic acid is introduced into an animal at an embryonic stage, and wherein said nucleic acid disrupts endogenous *MIF* gene activity wherein MIF protein production is prevented and wherein the animal, which is a homozygous MIF deficient mutant, is a suitable bioreactor for production of high affinity anti-MIF antibodies.

It is a further object of the invention to provide a transgenic animal genome comprising a homozygous disruption of the endogenous *MIF* gene, wherein said disruption comprises the insertion of a selectable marker sequence, and wherein said disruption results in said animal, which lacks an endogenous MIF as compared to a wild type animal and wherein said animal is a bioreactor for anti-MIF antibodies possessing high affinity.

Another object of the invention is to provide a method for producing a transgenic animal lacking endogenous MIF, said method comprising the steps of: (A) introducing a MIF targeting construct comprising a selectable marker sequence into a embryonic stem cell; (B) introducing said animal embryonic stem cell into a animal embryo; (C) transplanting said embryo into a pseudopregnant animal; (D) allowing said embryo to develop to term; and (B) identifying a transgenic animal whose genome comprises a disruption of the endogenous *MIF* gene at least one allele; (F) breeding the transgenic animal of step E to obtain a transgenic animal whose genome comprises a homozygous disruption of the endogenous *MIF* gene ($MIF^{-/-}$), wherein said disruptions results in an animal which lacks at least one endogenous MIF as compared to a wild type animal.

Brief Description of the Drawing Figures

Fig. 1 shows the generation of high affinity, anti-MIF Mabs in MIF gene knock out mice as assayed by ELISA of the first fusion which was produced by human MIF S/OVA immunized mice (E1).

Fig. 2 shows the generation of high affinity, anti-MIF Mabs in MIF gene 5 knockout mice as tested by ELISA of the second fusion, which was produced by

immunizing mice with solubler MIF. The antigen used in the ELISA for detection was biotin-MIF.

Fig. 3 shows that MIF catalyzes keto-enol tautomerase to tautomerize *p*-hydroxyphenylpyruvate.

Fig. 4 shows the MIF mediated signaling events that occur in the protein kinase A (PKA) and MAP kinase (MAPK) signaling cascade, as described by Mitchell *et al., J. Biol. Chem.* 274: 18100-6 (1999).

Fig. 5 depicts the transcription-based assay for determining anti-MIF antibody neutralization activity using the SRE-SEAP transcription and secretion assay.

Fig. 6 shows the percent inhibition of induced by anti-MIF antibodies on MIF induced SRE-SEAP transcription and secretion 10 :g/ml antibody and 10 :g/ml rMIF were used in each reaction.

Fig. 7 shows the anti-MIF Mab effects on MIF stimulated MMP-1 release from dermal fibroblasts.

Fig. 8 shows the anti-MIF Mab effects on VEGF-stimulated proliferation of human umbilical vein endothelist (HOVE) cells.

Fig. 9 shows anti-MIF Mab effects on MIF + LPS induced lethality in BALB/c mice when 10 mg LPS/kg body weight is administered per mouse.

Fig. 10 shows anti-MIF Mab effects on MIF + LPS induced lethality in BALB/c mice when 12.5 mg LPS/kg body weight is administered per mouse.

Fig. 11 shows anti-MIF Mab effects on MIF + LPS induced lethality in BALB/c mice when 15 mg LPS/kg body weight is administered per mouse.

Figs. 12A and 12B show the results of an assay that measured the effect of VEGF stimulation of HUVE cell proliferation over time in the absence of VEGF or at concentrations of 25 ng or 100 ng of VEGF over time.

Fig. 13 shows the results of an assay that evaluated effect of anti-MIF antibody on HOVE cells proliferation (various antibodies tested) at a concentration of 50 mg/ml in wells containing 625 cells/well after three (3) days.

Fig. 14 shows the results of an assay that, similar to the assay shown in Fig. 13, compares the effect of different anti-MIF antibodies at a concentration of 50 mg/ml on HUVE cell proliferation in microwells containing 2500 cells/well after five (5) days.

Fig. 15 shows the results of an assay that compares the effect of different anti-MIF antibodies on MIF-enhanced archidonic acid release in RAW264.7 cells transfected with the MIF gene (at antibody concentrations of 4 mg/ml and 20 mg/ml).

Fig. 16 contains an assay that compares binding of two lead candidate anti-MIF mabs, which were immobilized, particularly with respect to the capture of biotin-human MIF at different antibody concentrations.

Figs. 17 to 30 contain amino acid and DNA sequences for lead antibodies according to the invention.

Detailed Description of the Invention

A. Definitions

By "MIF" or "macrophage migration inhibitory factor" is meant the protein or nucleic acid encoding the protein which is responsible for attracting macrophages to a site. A preferred MIF is mammalian MIF, with most preferred being a human MIF. "MIF" also includes GIF (glycosylation-inhibiting factor), MIF-l, MIF-2, MIF-3, MIF-like proteins, and fragments of the MIF or MIF-like proteins. Additional forms of MIF encompassed by the term include those listed in Table 1, and as described in Weiser *et al.*, (1989) and U.S. Patent Application Nos. 08/243,342; 08/462,350; 08/462,350 and 08/602,929; in PCT applications WO 96/09384; WO 90/11301; WO 94/26923; WO 95/31468 (to MIF-3); and in U.S. Patent Nos. 5,328,990; 5,350,687; 4,299,814; 4,708,937 and European Patent No. 263072 (to macrophage inhibitory related peptides 8 and 14). The "MIF" proteins can also be in the form of a fusion protein.

By "knock-out animal," "KO animal," and "transgenic animal" is meant an animal in which a *MIF* gene has been functionally disrupted or inactivated. This inactivation refers to a modification of the gene in a manner which decreases or prevents expression of that gene and/or its product in a cell. The expression of the gene's product is completely suppressed. A functionally disrupted gene includes a modified gene which expresses a truncated polypeptide having less than the entire coding sequence of the wild-type gene.

By "animal" is meant to include preferably such mammals as primates, bovines, canines, felines, ovines, porcines, and rodents, *etc.* Preferable rodents include

mice, hamsters, rabbits and guinea pigs. However, animals can include any eukaryote.

By "antibody" is intended to refer broadly to any immunologic binding agent, such as IgG (including IgG_1, IgG_2, IgG_3, and IgG_4), 1gM, IgA, IgD, 1gE, as well as antibody fragments. As used herein, "isotype" refers to the antibody class *(e.g.*, IgM or IgG_1) that is encoded by heavy chain constant region genes. As used herein, "isotype switching" refers to the phenomenon by which the class, or isotype, of an antibody changes from one immunoglobulin (Ig) class to one of the other Ig classes. Antibodies in the broadest sense covers intact monoclonal antibodies, polyclonal antibodies, as well as biologically active fragments of such antibodies and altered antibodies.

By "monoclonal antibody" is meant an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al., Nature* 256: 495-7 (1975), or may be made by recombinant DNA methods (see, *e.g.*, U.S. Pat. No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described for example in Clackson *et al., Nature 352:* 624-8 (1991) and Marks *et al., J. Mol. Biol.*, 222: 581-97 (1991).

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with, or homologous to, corresponding sequences in antibodies derived from a

particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired therapeutic activity, *e.g.*, high affinity recognition of a MIF protein (U.S. Pat. No. 4,816,567; Morrison *et al., Proc. Natl. Acad. Sci. USA,* 81: 6851-5 (1984)).

"Humanized" forms of non-human *(e.g.,* murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies), which contain minimal sequence derived from a non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementarity-determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, rabbit or other mammal having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and optimize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc). For further details, see Jones *et al., Nature* 321: 522-5 (1986); Reichmann *et al., Nature* 332: 323-9 (1988); and Presta *Curr. Op. Struct. Biol.* 2: 593-6 (1992).

By "antibody fragment" or "immunogenic fragment" is meant an immunoglobulin, including segments of proteolytically-cleaved or recombinantly-prepared portions of an antibody molecule that are capable of selectively reacting with a particular antigen or antigen family *(e.g.,* MIF). Nonlimiting examples of such proteolytic and/or recombinant fragments include "Fab", "$F(ab')_2$", and "Fab"', "scFv" and "Fv" fragments. Recombinant techniques for producing Fv fragments are set forth in WO 88/0 1649, WO 88/-06630, WO 88/07085, WO 88/07086, and WO

88/09344. By a "V_H" fragment is meant that the variable region has at least a portion of a heavy chain variable region capable of being used as an antigen binding functionality. The preparation and use of a light chain variable region (VL) as an antigen binding functionality is set forth in an article by Williams *et al., Proc. Natl. A cad. Sci. (USA)* 86: 5537-41 (1989).

By "high-affinity antibody" is meant an antibody which binds to a MIF or GIF epitope with an affinity lower than 10^{-8} M *(e.g.,* 10^{-9}M, 10^{-10} M, *etc.)*. These antibodies should be capable of recognizing the native MIF or GIF epitopes, unlike MIF antibodies 15.5 and 3D9, which recognize primarily denatured MIF with only weak recognition of native, undenatured MIF. Available antibodies against MIF include XIV 15.5 and 3D9. These all exhibit affinities less than 10^{-6}M against native, soluble MIF protein. As a result, the *in vivo* biological potency is weak and is achieved at 20-3 0 mg/kg of antibody, which is too high for medical usage. Accordingly, the anti-MIF or anti-GIF antibodies produced by the knock-out animal will preferably yield a therapeutic response in a human when administered at dosages of about to about 15 mg/kg or less.

By "nucleic acid" is meant to include DNA, genomic DNA, RNA, mRNA and cDNA. The preferred nucleic acids of the invention include those that encode immunoglobulins or fragments thereof which recognize MIF. The term also may encompass a MIF targeting construct for the purpose of making a $MIF^{-/-}$ mouse.

By "gene" is meant the segment of DNA involved in producing a polypeptide chain. It includes regions preceding and following the coding region, as well as intervening sequences *(e.g.,* introns) between the coding sequences (exons).

By "homologous recombination" is meant the process by which a nucleic acid molecule with similar genetic information aligns itself with a second nucleic acid molecule and exchanges nucleotide strands. A nucleotide sequence of the recombinant nucleic acid which is effective to achieve homologous recombination at a predefined position of a target nucleic acid therefore indicates a nucleotide sequence which facilitates the exchange of nucleotides strands between the recombinant nucleic acid molecule at a defined position of a target gene. The effective nucleotide sequence generally comprises a nucleotide sequence which is complementary to a desired target nucleic acid molecule *(e.g.,* the gene locus to be modified), thus promoting nucleotide base pairing. Any nucleotide sequence can be employed as

long as it facilitates homologous recombination at a specific and selected position along the target nucleic acid molecule *(e.g.,* a gene encoding a MIF protein).

By "not functional" or "functionally inactive" is meant that the MIF protein is not operational or the *MIF* gene cannot synthesize a functional MIF protein.

"Expression vector" is given a functional definition, and any DNA sequence which is capable of effecting expression of a specified DNA code in a suitable host is included in this term. As at present, such vectors are frequently in the form of plasmids, thus "plasmid" and "expression vector" are often used interchangeably. However, the invention is intended to include such other forms of expression vectors which serve equivalent functions and which may, from time to time, become known in the art. Typically an "expression vector" is a nucleic acid molecule comprising (1) a promoter and other sequences *(e.g.,* leader sequences) necessary to direct expression of a desired gene or DNA sequence, and (2) the desired gene or DNA sequence. Optionally, the nucleic acid molecule may comprise a poly A signal sequence to enhance the stability of the gene transcript and/or to increase gene transcription and expression.

"Transformation" refers to the introduction of DNA into a recipient host cell that changes the genotype and consequently results in a change in the recipient cell. "Transformation" and "transfection" are often used interchangably.

"Host cells" refers to cells which have been recombinantly transformed with vectors constructed using recombinant DNA techniques. One preferred host cell, may be a $MIF^{-/-}$ deficient cell. A less preferred host cell is one in which the cell is $MIF^{-/+}$. Additionally, host cells may also be those cells transfect with a nucleic acid encoding an immunoglobulin derived from a $MIF^{-/-}$ of the invention.

In descriptions of processes for isolation of antibodies from recombinant hosts, the terms "cell," "cell culture" and "cell line" are used interchangeably to denote the source of antibody, unless it is clearly specified otherwise. In other words, recovery of antibody from the "cells" may mean either from spun down whole cells, or from the cell culture containing both the medium and the suspended cells.

By "purified" and "isolated" is meant, when referring to a polypeptide or nucleotide sequence, that the indicated molecule is present in the substantial absence of other biological macromolecules of the same type. The term "purified" as used herein preferably means at least 75% by weight, more preferably at least 85% by

weight, more preferably still at least 95% by weight, and most preferably at least 98% by weight, of biological macromolecules of the same type are present. An "isolated nucleic acid molecule" which encodes a particular polypeptide refers to a nucleic acid molecule which is substantially free of other nucleic acid molecules that do not encode the subject polypeptide; however, the molecule may include some additional bases or moieties which do not deleteriously affect the basic characteristics of the composition. Thus, for example, an isolated nucleic acid molecule which encodes a particular CDR polypeptide consists essentially of the nucleotide coding sequence for the subject molecular recognition unit.

By "modulating" or "regulating" is meant the ability of an agent to alter from the wild-type level observed in the individual organism the wild-type activity of a MIF. MIF activity can be regulated at transcription, translation, nucleic acid or protein stability or protein activity.

B. *Method of Preparing a Knock-Out Mouse or Other Transgenic Animal*

Transgenic animals typically can be prepared by homologous recombination. Gene deletion or knockout can be performed as described by Capecchi, *Science* 244: 1288-92 (1982); Brinster, *Int. J. Dev. Biol.* 37: 89-99 (1993); and DOETSCHMAN, IN TRANSGENIC ANIMAL TECHNOLOGY: A LABORATORY HANDBOOK 115-146 (C. A. Pinkert *et al.*, ed., 1994). Knock-out animals can be prepared using embryonic stem (ES) cells or ES-like cells.

C. *ES Cells*

The genome of ES cells can be manipulated *in vitro* by introducing a desired foreign DNA by such techniques as electroporation, microinjection, precipitation reactions, transfection or retroviral insertion (Bradley *et al., Nature* 309: 255-6 (1984); Gossler *et al., Proc. Natl. Acad. Sci. U.S.A.* 83: 9065-9 (1986); ROBERTSON ET AL., TERATOCARCINOMAS AND EMBRYONIC STEM CELLS: A PRACTICAL APPROACH (1987); Kuehn *et al., Nature* 326: 295-8 (1987); Thompson *et al., Cell 56:* 313-21 (1989); Zimmer *et al., Nature* 338: 150-3 (1989); and Doetschman (1994).

ES-like cell lines have been identified and can be used as described by:

Hamsters Doetschman *et al., Dev. Biol.* 127: 224-7 (1988)

	Pigs	Notarianni *et al., J. Reprod. Fertil. Suppl.* 41: 51-6 (1990); Piedrahita *et al., Theriogenology* 34: 879-90 1 (1990); and Strojek *et al., Theriogenology* 33:901-13 (1990)
	Sheep	Piedrahita *et al.* (1990).

Other animals and methods of obtaining transgenic animals, using methods other than ES cells or ES-like cells, include those of Iannaccone *et al., Dev. Biol.* 163: 288-92 (1994) for rats; Stice *et al., Theriogenology* 41: 301 (Abstract) (1994) for bovine fetuses, and Wheeler, *J. Reprod. Fertil.* 6 (Suppl.): 1-6 (1994) and Gerfen *et al., Anim. Biotech.* 6: 1-14 (1995) for pigs. The methods by Cherney *et al., Theriogenology* 41: 175 (1994) can be used for culturing bovine primordial germ cell-derived cell lines in culture. In addition to ES or ES-like cells, inner cell mass cells of blastocysts from animals such as bovines can be used as described by Van-Stekelenburg-Hamers *et al., Mol. Reprod. Dev.* 40: 444-54 (1995) and Collas *et al., Mol. Reprod. Dev.* 38: 264-7 (1994).

D. *Nuclear Transfer*

Homologous recombination events can also be used with nuclear transfer or transplantation. Using this technique eliminates the need for ES or ES-like cell lines. Nuclear transfer ca be performed using the methods described by Campbell *et al., Nature* 380: 64-8 (1996).

E. *Homologous Recombination*

In one aspect of the invention, a targeting vector is employed to insert a selectable marker into a predefined position of a gene *(e.g.,* the gene encoding a MIF protein). The position is selected to achieve functional disruption of the gene upon insertion of the selectable marker. For such purposes, a preferred embodiment is a recombinant nucleic acid molecule comprising: (1) a 5' nucleotide sequence that is effective to achieve homologous recombination at a first predefined position of a mammalian *MIF* gene operably linked to (2) the 5' terminus of a first selectable nucleotide sequence which confers a first selection characteristic on a cell in which it is present, and (3) a 3' nucleotide sequence which is effective to achieve homologous recombination at a second predefined position of the *MIF* gene, operably linked to the

3' terminus of the first selectable nucleotide sequence. The recombinant nucleic acid molecule is effective to achieve homologous recombination in a mammalian chromosome at predefined location, which contains a gene encoding a MIF protein. Fragments of the targeting vector are also within the scope of the invention, *e.g.*, recombinant nucleic acid molecules comprising elements (1) and (2), or comprising elements (2) and (3), *etc.*

Any nucleotide sequence can be employed, as long as it facilitates homologous recombination at a specific and selected position along the target nucleic acid molecule. Generally, there is an exponential dependence of targeting efficiency on the extent or length of homology between the targeting vector and the target locus. Selection and use of sequences effective for homologous recombination is described, *e.g.*, in Deng *et al., Mol. Cell. Bio.* 12: 3365-71 (1992); Bollag *et al., Annu. Rev. Genet.* 23: 199-225 (1989); Waldman *et al., Mol. Cell. Bio.* 8: 5350-7 (1988).

An aspect of the present invention is to suppress or functionally disrupt expression of a *MIF gene.* The phrases "disruption of the gene", "gene disruption," "suppressing expression," "gene suppression," "functional inactivation of the gene," or "functional gene inactivation" refer to modification of the gene in a manner which prevents expression of that gene and/or its product *(e.g.*, a MIF protein) in a cell. The expression of the gene's product is completely suppressed. A functionally disrupted gene, *e.g.*, a functionally disrupted *MIF* gene, includes a modified gene that expresses a truncated MIF polypeptide having less than the entire coding sequence of the wild-type *MIF* gene. A gene can also be functionally disrupted by affecting its mRNA structure in such a way to create an untranslatable message, *e.g.*, frame-shift, decreased stability, *etc.*

In accordance with the present invention, a *MIF* gene is modified in such a manner which is effective to disrupt expression of the corresponding gene product. Thus, a functionally disrupted recombinant *MIF* gene does not express a functional MIF polypeptide or expresses a functional MIF polypeptide at levels which are substantially less than wild-type levels of MIF. The gene can be modified in any effective position, *e.g.*, enhancers, promoters, regulatory regions, noncoding sequences, coding sequences, introns, exons, *etc.*, so as to decrease or prevent expression of that gene in a cell. Insertion into a region of a *MIF* gene, *e.g.*, a MIF-1, MIF-2 or MIF-3 gene, is usually accomplished by homologous recombination. A

recombinant nucleic acid molecule comprising regions of gene homology and a nucleotide sequence coding for a selectable marker gene is inserted into the promoter and/or coding region and/or noncoding regions of a *MIF* gene, whereby expression of the gene is functionally disrupted. When this knockout construct is then inserted into a cell, the construct can integrate into the genomic DNA. Thus, progeny of the cell will only express only one functional copy of the gene; the other copy will no longer express the gene product, or will express it at a decreased level, as the endogenous nucleotide sequence of the gene is now disrupted by the inserted nucleotide sequence. If desired, the functional gene can be inactivated in a second analogous step.

The nucleotide sequence effective for homologous recombination is operably linked to a nucleotide sequence, preferably a selectable marker nucleotide sequence or gene, which is to be inserted into the desired target nucleic acid. For example, an aspect of the present invention is to replace all or part of the nucleotide coding sequence for a MIF protein, with a nucleotide sequence for a selectable marker.

The recombinant nucleic acid is preferably inserted into a cell with chromosomal DNA that contains the endogenous gene to be knocked out. In the cell, the recombinant nucleic acid molecule can integrate by homologous recombination with the DNA of the cell in such a position so as to prevent or interrupt transcription of the gene to be knocked out. Such insertion usually occurs by homologous recombination *(i.e.,* regions of the targeting vector that are homologous or complimentary to endogenous DNA sequences hybridize to each other when the targeting vector is inserted into the cell; these regions can then recombine so that part of the targeting vector is incorporated into the corresponding position of the endogenous genomic DNA).

As discussed, one or more nucleotide sequences can be inserted into a *MIF* gene to suppress its expression. It is desirable to detect the presence of the nucleotide sequence in the gene. Such detection can be accomplished in various ways, including by nucleic acid hybridization *(e.g.,* Northern or Southern blot), antibody binding to a protein epitope encoded by the inserted nucleic acid, or by selection for a phenotype of the inserted sequence. Accordingly, such an inserted nucleotide sequence can be referred to as a first selectable nucleotide sequence. A first selectable nucleotide sequence preferably confers a first selection characteristic on a cell in which it is present. By the phrase "selection characteristic," it is meant, *e.g.,* a characteristic

which is expressed in a cell and which can be chosen in preference to another or other characteristics. The selectable nucleotide sequence, also known as selectable marker gene, can be any nucleic acid molecule that is detectable and/or assayable after it has been incorporated into the genomic DNA of the mammal. The selection characteristic can be a positive characteristic, *i.e.*, a characteristic which is expressed or acquired by cells and whose presence enables selection of such cells. A positive selection characteristic can enable survival of the cell or organism, *e.g.*, antibiotic resistance, ouabain-resistance (a gene for an ouabain-resistant sodium/potassium ATPase protein). Examples of positive selection characteristics and a corresponding selection agent include, *e.g.*, *Neo* and G4 18 or kanomycin; Hyg and hygromycin; hisD and histidinol; Gpt and xanthine; Ble and bleomycin; and Hprt and hypoxanthine. See, *e.g.*, U.S. Pat. No. 5,464,764 and Capecchi, *Science* 244: 1288-92 (1989). The presence of the selectable gene in the targeted sequence can also be identified by using binding ligands which recognize a product of the selectable gene, *e.g.*, an antibody can be used to identify a polypeptide product coded for by the selectable gene, an appropriate ligand can be used to identify expression of a receptor polypeptide encoded by the selectable gene, or by assaying for expression of an enzyme encoded by the selectable gene. Preferably, the selectable marker gene encodes a polypeptide that does not naturally occur in the mammal.

The selectable marker gene can be operably linked to its own promoter or to another promoter from any source that will be active or can easily be activated in the cell into which it is inserted. However, the selectable marker gene need not have its own promoter attached, as it may be transcribed using the promoter of the gene into which it is inserted. The selectable marker gene can comprise one or more sequences to drive and/or assist in its expression, including, *e.g.*, ribosome-recognition sequences, enhancer sequences, sequences that confer stability to the polypeptide or RNA, and/or a polyA sequence attached to its 3' end to terminate transcription of the gene. A positive selectable marker facilitates selection for recombinants in which the positive selectable marker has integrated into the target nucleic acid by homologous recombination. A gene targeting vector in accordance with the present invention can also further comprise a second selection characteristic encoded by a second selectable gene to further assist in the selection of correctly targeted recombinants. A negative selection marker permits selection against cells in which only non-homologous

recombination has occurred. In one preferred embodiment, the second selectable marker gene confers a negative selection characteristic upon a cell in which it has been introduced. Such negative selection characteristics can be arranged in the targeting vector in such a way to facilitate discrimination between random integration events and homologous recombination. By the term "negative selection", it is meant a selection characteristic which, when acquired by the cell, results in its loss of viability *(i.e.,* it is lethal to the cell). A nucleoside analog, gancyclovir, which is preferentially toxic to cells expressing HSV tk (herpes simplex virus thymidine kinase), can be used as a negative selection agent, as it selects for cells which do not have an integrated HSV tk selectable marker. FIAU (1,2-deoxy-2-fluoro-∀-d-arabinofuransyl-5-iodouracil) can also be used as a negative selection agent to select for cells lacking HSV tk. Other negative selectable markers can be used analogously. Examples of negative selection characteristics and a corresponding enzyme include thymidine kinase (HSV tk) and acyclovir, gancyclovir, or FIAU; Hprt and 6-thioguanine or 6-thioxanthine; diphtheria toxin; ricin toxin; cytosine deaminase and fluorocytosine.

The negative selectable marker is typically arranged on the gene targeting vector 5' or 3' to the recombinogenic homology regions so that double-crossover replacement recombination of the homology regions transfers the positive selectable marker to a predefined location on the target nucleic acid, but does not transfer the negative selectable marker. For example, a tk cassette can be located at the 3' end of a murine *MIF* gene, about 150 base pairs from the 3' stop codon. More than one negative selectable marker can also be utilized in a targeting vector. The positioning of, for example, two negative selection vectors at the 5' and 3' ends of a targeting vector further enhances selection against target cells which have randomly integrated the vector. Random integration sometimes results in the rearrangement of the vector, resulting in excision of all or part of the negative selectable marker prior to the random integration event. When this occurs, negative selection cannot be used to eliminate those cells which have incorporated the targeting vector by random integration rather than homologous recombination. The use of more than one negative selectable marker substantially enhances the likelihood that random integration will result in the insertion of at least one of the negative selectable

markers. For such purposes, the negative selectable markers can be the same or different.

The use of a positive-negative selection scheme reduces the background of cells having incorrectly integrated, targeted construct sequences. Positive-negative selection typically involves the use of two active selectable markers: (1) a positive selectable marker *(e.g., neo)* that can be stably expressed following random integration or homologous targeting, and (2) a negative selectable marker *(e.g., tk)* that can only be stably expressed following random integration. By combining both positive and negative selection, host cells having the correctly targeted homologous recombination event can be efficiently obtained. Positive-negative selection schemes can be performed as described in, *e.g.*, U.S. Pat. No. 5,464,764; and WO 94/06908. It is recognized, however, that one or more negative selectable markers are not required to carry out the present invention, *e.g.*, produce a transgenic animal in which a *MIF* gene is functionally inactivated or disrupted.

A recombinant nucleic acid molecule according to the present invention can also comprise all or part of a vector. A vector is, *e.g.*, a nucleic acid molecule which can replicate autonomously in a host cell, *e.g.*, containing an origin of replication. Vectors can be useful to perform manipulations, to propagate, and/or obtain large quantities of the recombinant molecule in a desired host. A skilled worker can select a vector depending on the purpose desired, *e.g.*, to propagate the recombinant molecule in bacteria, yeast, insect, or mammalian cells. The following vectors are provided by way of example. Bacterial: pQE70, pQE60, pQE-9 (Qiagen), pBS, pD10, Phagescript, phiX174, pBK Phagemid, pNH8A, pNH16a, pNH18Z, pNH46A (Stratagene); Bluescript KS+II (Stratagene); ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: PWLNEO, pSV2CAT, pOG44, pXT1, pSG (Stratagene), pSVK3, PBPV, PMSG, pSVL (Pharmacia). However, any other vector, *e.g.*, plasmids, viruses, or parts thereof, may be used as long as they are replicable and viable in the desired host. The vector can also comprise sequences that enable it to replicate in the host whose genome is to be modified. The use of such vector can expand the interaction period during which recombination can occur, increasing the targeting efficiency.

In accordance with an aspect of the present invention, the function of a *MIF* gene, such as MIF-1, is disrupted or knocked out by the insertion of an exogenous or

heterologous sequence into it, interrupting its function. For example, the exogenous or heterologous sequence can be inserted into a region of the gene, such as MIF-1 before its first start codon. The nucleotide sequence coding for a selectable characteristic can be inserted into the *MIF* gene in such a manner by homologous recombination so that it is operably linked to an endogenous *MIF* gene promoter. Upon integration of the selectable marker gene into the desired predefined position of the *MIF* gene, expression of the selectable characteristic is driven by the endogenous *MIF* gene promoter, permitting its detection in those cells in which the construct has integrated.

The selectable marker gene can also be integrated at positions downstream of (3' to) the first start codon of the *MIF* gene. The *MIF* gene can be integrated out-of-reading frame or in-reading frame with the MIF polypeptide so that a fusion polypeptide is made, where the fusion polypeptide is less active than the normal product. By detecting only those cells which express the characteristic, cells can be selected which contain the integrated sequence at the desired location. A convenient way of carrying out such selection is using antibiotic resistance. As described herein, neomycin resistance is utilized as the selectable characteristic. Cells grown in the presence of a toxic concentration of G418 will normally die. Acquisition of the neomycin resistance gene *(neo)* by homologous recombination rescues cells from the lethal effect, thereby facilitating their selection.

The *MIF* gene is knocked-out or functionally interrupted by the integration event. The insertion of the selectable gene ahead of the MIF coding sequence effectively isolates it from a promoter sequence, disabling its expression. If the selectable gene contains a transcription terminator, then transcription of the gene using the MIF promoter will terminate immediately after it and will rarely result in the transcription of a MIF coding sequence. The *MIF* gene can also be knocked out by a deletion without a replacement, such as a site-directed deletion of a part of the gene. Deleted regions can be coding regions or regulatory regions of the gene.

A *MIF* gene can be modified at any desired position. It can be modified so that a truncated MIF polypeptide is produced having one or more activities of the complete MIF polypeptide. As already discussed, such a modified gene is a functionally disrupted gene.

If desired, the insertion(s) can be removed from the recombinant gene. For example, a neomycin cassette can replace exons of a mouse *MIF* gene to functionally inactivate it. The neomycin cassette can be subsequently removed from the *MIF* gene, *e.g.*, using a recombinase system. The Cre-lox site specific recombination system is especially useful for removing sequences from a recombinant gene. To utilize the Cre-lox system, recombinase recognition sites are integrated into the chromosome along with the selectable gene to facilitate its removal at a subsequent time. For guidance on recombinase excision systems, see, *e.g.*, U.S. Pat. Nos. 5,626,159, 5,527,695, and 5,434,066. See also, Orban *et al., Proc. Natl. Acad. Sci. USA* 89: 6861-5 (1992); O'Gorman *et al., Science* 251: 1351-5 (1991); and Sauer et al., *Nuc. Acids Res.* 17: 147-61 (1989).

A nucleic acid comprising a nucleotide sequence coding without interruption means that the nucleotide sequence contains an amino acid coding sequence for a polypeptide, with no non-coding nucleotides interrupting or intervening in the coding sequence, *e.g.*, absent intron(s) or the noncoding sequence, as in a cDNA.

Another aspect of the present invention relates to host cells comprising a recombinant nucleic acid of the invention. A cell into which a nucleic acid is introduced is a transformed cell. Preferred nucleic acids include the knock-out cassettes described above, as well as nucleic acids encoding a high affinity antibody or fragment thereof which is produced by a $MIF^{-/-}$ knockout animal. Host cells include, mammalian cells, *e.g.*, murine Ltk-, murine embryonic stem cells, COS-7, CHO, HeLa, insect cells, such as Sf9 and Drosophila, bacteria, such as *E. coli, Streptococcus,* bacillus, yeast, fungal cells, plants, embryonic stem (ES) cells *(e.g.,* mammalian, such as mouse), neuronal cells (primary or immortalized), *e.g.*, NT-2, NT-2N, PC-12, SY-5Y, neuroblastoma. See, also Goeddel, *Methods in Enzymology* 185: 3-7 (1990) A nucleic acid can be introduced into the cell by any effective method including, *e.g.*, calcium phosphate precipitation, electroporation, injection, pressure, DEAE-Dextran mediated transfection, fusion with liposomes, and viral transfection. When the recombinant nucleic acid is present in a mouse cell, it is preferably integrated by homologous recombination into the mouse cell gene locus. Additional methods are as described in SAMBROOK *ET AL.*, MOLECULAR CLONING: A LABORATORY MANUAL (1989).

A transformed cell can contain a recombinant gene integrated into its chromosome at the targeted gene locus. A targeting vector which comprises sequences effective for homologous recombination at a particular gene locus, when introduced into a cell under appropriate conditions, will recombine with the homologous sequences at the gene locus, introducing a desired selectable gene into it. When recombination occurs such that insertion results, the nucleic acid is integrated into the gene locus. The gene locus can be the chromosomal locus which is characteristic of the species, or it can be a different locus, *e.g.*, translocated to a different chromosomal position, on a supernumerary chromosome, on an engineered "chromosome," *etc.*

As discussed below, the present invention also relates to transgenic animals containing one or more modified *MIF* genes. The transgenic animals produced in accordance with the present invention can be used as a source to establish primary or established, *e.g.*, immortalized, cell lines according to various methods as the skilled worker would know. Since the animals (either homozygotes or heterozygotes) contain a modified *MIF* gene, the corresponding cell lines would be expected to have the same genotype. The cell lines can be derived from any desired tissue or cell-type, including, *e.g.*, liver, epithelia, neuron, fibroblast, mammary, lung, kidney, pancreas, stomach, thyroid, prostate, osteoblasts, osteoclasts, osteocytes, osteoprogenitor cells, muscle *(e.g.*, smooth), *etc.*

Cell lines produced in accordance with the present invention are useful for a variety of purposes. In one aspect of the invention, it is desirable to create panels of cell lines which differ in the expression of one or more genes. For example, the present invention describes and enables the production of cell lines which lack a *MIF* gene, such as the *MIF-1* gene. A MIF-functionally-disrupted cell line differs from the parental *(i.e.*, starting) cell line by the expression of the *MIF* gene. The availability of such pairs of cell lines, *i.e.*, plus or minus for MIF expression (or any other desired gene, *e.g.*, *MIF-2),* is useful to distinguish the effects of MIF from those of other *MIF* genes products. A cell line functionally-disrupted in one or more desired proteases *(e.g.*, MIF -1, MIF-2, *etc.),* in combination with the parental cell line intact for other MIF or MIF-like proteins, can be employed to specifically distinguish its activity *(e.g.*, MIF-1) from all other MIF proteins. Such genetic dissection can be used to develop, *e.g.*, drugs and therapeutics which target a specific gene product.

Gene functionally-disrupted cell lines can also be utilized to produce transgenic, either chimeric, heterozygous, or homozygous, animals, *e.g.*, non-human mammals. Such transgenic animals are useful as models to study the physiological role of a desired gene and to identify agents which specifically target the desired gene or a biological pathway in which it acts. Thus, an aspect of the invention is method of administering to a mammal functionally-disrupted for a *MIF* or MIF-like gene, *e.g.*, MIF, an amount of an agent effective to restore MIF activity.

The present invention also relates to a non-human transgenic animal, preferably a mammal, more preferably a mouse, which comprises a macrophage *MIF* gene, which has been engineered employing a recombinant nucleic acid according to the present invention. Generally, a transformed host cell, preferably a totipotent cell, whose endogenous gene has been modified using a recombinant nucleic acid as described above is employed as a starting material for a transgenic embryo. The preferred methodology for constructing such a transgenic embryo involves transformed embryonic stem (ES) cells prepared as described herein employing a targeting vector comprising a recombinant nucleic acid according to the invention. A particular gene locus, *e.g.*, *MIF-1*, is modified by targeted homologous recombination in cultured ES or ES-like cells employing a targeting vector comprising a recombinant nucleic acid according to the invention. The ES or ES-like cells are cultured under conditions effective for homologous recombination. Effective conditions include any culture conditions which are suitable for achieving homologous recombination with the host cell chromosome, including effective temperatures, pH, medias, additives to the media in which the host cell is cultured *(e.g.*, for selection, such as G418 and/or FIAU), cell densities, amounts of DNA, culture dishes, *etc.* Cells having integrated the targeting vector are selected by the appropriate marker gene present in the vector. After homologous recombination has been accomplished, the cells contain a chromosome having a recombinant gene. In a preferred embodiment, this recombinant gene contains a positive selectable marker gene fused to endogenous *MIF* gene sequences. The transformed or genetically modified ES or ES-like cells can be used to generate transgenic non-human mammals, *e.g.*, mice, by injection into blastocysts and allowing the chimeric blastocysts to mature, following transfer into a pseudopregnant mother. See, *e.g.*, TERATOMACARCINOMA AND EMBRYONIC STEM CELLS: A PRACTICAL APPROACH (E. J. Robertson, ed., IRL Press). Various stem

cells can be used, as known in the art, *e.g.*, AB-l, HM-l D3, CCl.2, E-14T62a, or RW4. Offspring born to foster mothers may be screened initially for mosaic coat color, where a coat color selection strategy has been employed. Alternatively, DNA from tail or other suitable tissue of the offspring can be used to screen for the presence of the DNA targeting vector. Offspring that appear to be mosaics are then crossed to each other, if it believed they carry the modified gene in their germ line, in order to generate MIF deficient homozygotes. See, *e.g.*, U.S. Pat. Nos. 5,557,032 and 5,532,158.

In addition to the ES or ES-like cell methods described herein, transgenic animals can be created by other methods, *e.g.*, by pronuclear injection of recombinant genes into pronuclei of one-cell embryos, incorporating an artificial yeast chromosome into embryonic stem cells, gene targeting methods and embryonic stem cell methodology. See, *e.g.*, U.S. Pat. Nos. 4,736,866; 4,873,191; 4,873,316; 5,082,779; 5,304, 489; 5,174, 986; 5,175, 384; 5,175,385; and 5,221,778; and Gordon *et al., Proc. Natl. Acad. Sci. U.S.A.*, 77: 7380-4 (1980); Palmiter *et al., Cell* 41: 343-5 (1985); Palmiter *et al., Ann. Rev. Genet.* 20: 465-99 (1986); Askew *et al., Mol. Cell. Bio.* 13: 4115-24 (1993); Games *et al., Nature* 373: 523-7 (1995); Valancius *et al., Mol. Cell. Bio.* 11: 1402-8 (1991); Stacey *et al., Mol. Cell. Bio.* 14:1009-16 (1994); Hasty *et al., Nature* 350: 243-6 (1995); and Rubinstein *et al., Nucl. Acid Res.* 21: 2613-7 (1993).

As discussed, one aspect of the invention relates to a knock-out mammal, such as a mouse, comprising cells which contain at least one functionally disrupted, recombinant *MIF* gene *(e.g.*, heterozygous or homozygous) at a chromosomal *MIF* gene locus. The cells and animals can be created in accordance with the examples below by inserting an exogenous nucleotide sequence into the *MIF* gene. However, other methods can be used to create a functionally interrupted gene. For example, a termination codon can be inserted into a *MIF* gene, using, *e.g.*, a replacement type vector as described in Rubinstein *et al., Nucleic Acid Res.* 21: 2613-7 (1993) or a tag-and-exchange strategy as described in Askew *et al., Mol. Cell. Bio.* 13: 4115-24 (1993), *etc.* Functional interruption of a *MIF* gene can also be achieved classically by mutagenesis, such as chemical or radiation mutagenesis.

A recombinant nucleic acid molecule according to the present invention can be introduced into any non-human mammal, including a mouse (HOGAN ET AL.,

MANIPULATING THE MOUSE EMBRYO: A LABORATORY MANUAL (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1986)), pig (Hammer *et al., Nature* 315: 343-5 (1985)), sheep (Hammer *et al., Nature* 315: 343-345 (1985)), cattle, rat, or primate. See also, *e.g.*, Church, *Trends in Biotech.* 5: 13-9 (1987); Clark *et al., Trends in Biotech.* 5: 20-4 (1987); DePamphilis *et al., BioTechniques* 6: 662-80 (1988); and STRATEGIES IN TRANSGENIC ANIMAL SCIENCE (Glenn M. Monastersky and James M. Robl, eds. 1995).

In the examples below, a murine *MIF* gene is modified by homologous recombination utilizing a gene targeting vector comprising regions of the murine *MIF* gene. To carry out genetic modification of another mammalian *MIF* gene, *e.g.*, a rat or a primate, it may be desirable to obtain analogous regions of the target *MIF* gene. A *MIF* gene from another species, using a murine or human *MIF* gene, can be accomplished by various methods known in the art, *e.g.*, PCR using a mixture of oligonucleotides based on a consensus sequence or MIF *(e.g.,* Leytus *et al., Biochemistry* 27: 1067-74 (1988)), nucleic acid hybridization using oligonucleotides, cDNA, *etc.*, at a desired stringency *(e.g.,* SAMBROOK ET AL., MOLECULAR CLONING, 1989).

A transgenic animal according to the present invention can comprise one or more *MIF* genes which have been modified by genetic engineering. For example, a transgenic animal comprising a *MIF* gene which has been modified by targeted homologous recombination in accordance with the present invention can comprise other mutations, including modifications at other gene loci and/or transgenes. Modifications to these gene loci and/or introduction of transgenes can be accomplished in accordance with the methods of the present invention, or other methods as the skilled worker would know. For instance, double-mutants can be made by conventional breeding, *i.e.*, crossing animals and selecting for a desired phenotype and/or genotype. In one embodiment of the invention, a transgenic animal can be constructed having at least a defective *MIF-1* gene *(e.g.,* a knock-out) and one or more other *MIF* or MIF-like genes coding for a MIF or MIF-like protein. In a preferred embodiment, the latter genes are null or functionally-disrupted. Such an animal can be homozygous *(-/-)* or heterozygous *(-/+)* for the desired loci, or a combination thereof.

For other aspects of the nucleic acids, polypeptides, antibodies, *etc.*, reference is made to standard textbooks of molecular biology, protein science, and immunology. See, *e.g.*, Davis *et al.*, BASIC METHODS IN MOLECULAR BIOLOGY (Elsevir Sciences Publishing, Inc., New York 1986); Hames *et al.*, NUCLEIC ACID HYBRIDIZATION (IL Press 1985), SAMBROOK *ET AL.*, (1989); CURRENT PROTOCOLS IN PROTEIN SCIENCE (F.M. Ausubel *etal.*, eds. John Wiley & Sons, Inc.), CURRENT PROTOCOLS IN HUMAN GENETICS (Nicholas C. Dracopoli *et al.*, eds. John Wiley & Sons, Inc. 1994); CURRENT PROTOCOLS IN PROTEIN SCIENCE (John E. Coligan *et al.*, eds. John Wiley & Sons, Inc. 1995); and CURRENT PROTOCOLS IN IMMUNOLOGY (John E. Coligan *et al.*, ed. John Wiley & Sons, Inc. 1991).

F. Method of Raising Antibodies in a Knock-Out Animal

Antibodies can be obtained from the blood serum of a $MIF^{-/-}$ animal immunized with a MIF antigen.

i. MIF Antigen

The MIF antigen used to raise antibodies can be from a complete MIF protein from any species, fragments thereof and fusion proteins containing all or a portion of a MIF protein. MIF sequences include, but at not limited to, any of the following:

TABLE 1

Name	GenPept Accession No.	Publication or Deposit
L-dopachrome-methyl ester tautomerase (macrophage MIF homolog) of *Trichuris trichiura*	P81748	
D-dopachrome tautomerase (murine)	O35215	Esumi *et al.*, *Mamm.* Genome 9: 753-7 (1998).
Macrophage MIF homolog (BMMIF) (*Brugia malayi*)	P91850	Pastrana *et al.*, *Infect. Immun.* 66: 5955-63 (1998).
L-Dopachrome-methyl ester tautomerase (macrophage MIF	P81529	Pennock *et al.*, *Biochem. J.* 335: 495-8 (1998).

Name	GenPept Accession No.	Publication or Deposit
homolog) *Trichinella spiralis*		
L-Dopachrome-methyl ester tautomerase (macrophage MIF homolog) *Trichuris muris*	P81530	Pennock *et al.*, (1998)
MIF-Like Protein C52E4.2. *Caenorhabditis elegans*	Q18785	
Macrophage MIF (Glycosylation-inhibiting factor) (GIF). *Sus scrofa*	P80928	
Macrophage MIF (Glutathione-binding 13 kD Protein). *Rattus norvegicus*	P30904	Sakai *et al., Biochem. Mol. Biol. Int.* 33: 439-46 (1994)
Macrophage MIF (Glycosylation-inhibiting factor, GIF).; *Homo sapiens*	P14174	Weiser *et al., Proc. Natl. Acad. Sci.* U.S.A. 86: 7522-6 (1989); Mikayama *et al., Proc. Natl. Acad. Sci.* U.S.A. 90: 10056-60 (1993); *Kato et al., Proc. Natl. Acad. Sci.* U.S.A. 93: 3007-10 (1996)
Macrophage MIF(P12A). Bovine	P80177	Galat *et al., Eur. J. Biochem.* 224: 417-21 (1994).
Macrophage MIF (delayed early response protein 6, DER6) (Glycosylation-inhibiting factor). Murine	P34884	Bernhagen *et al., Nature 365*: 756-9 (1993); Mikayama *et al.*, (1993).
Macrophage MIF. *Gallus gallus*	Q02960	Wistow *et al., Proc. Natl. Acad. Sci. U.S.A.* 90: 1272-5 (1993).
Chain B, Macrophage MIF Y95f Mutant. *Mus musculus*	5822094	
Chain A, Macrophage MIF Y95f Mutant. *Mus musculus*	5822093	
Chain C, Macrophage MIF Y95f Mutant.. *Mus musculus*	5822092	
Macrophage MIF. *Sus scrofa*	AAD50507	Abraham *et al., Domest. Anim. Endocrinol.* 15: 389-6 (1998).
Macrophage MIF (glycosylation-inhibiting factor). *Homo sapiens*	4505185	Mikayama *et al.*, (1993); Paralkar *et al., Genomics* 19: 48-51 (1994); Kozak *et al.*,

Name	GenPept Accession No.	Publication or Deposit
		Genomics 27: 405-11 (1995); Budarf *et al.*, *Genomics* 39: 235-6 (1997).
Chain C, Macrophage MIF with Pro-1 Mutated To Gly-1. *Homo sapiens.*	5542327	Lubetsky *et al.*, *Biochemistry* 38: 7346-54 (1999).
Chain B, Macrophage MIF with Pro-1 Mutated To Gly-1. *Homo sapiens.*	5542326	Lubetsky *et al.*, (1999).
Chain A, Macrophage MIF with Pro-1 Mutated To Gly-1. *Homo sapiens*	5542325	Lubetsky *et al.*, (1999).
Chain C, Macrophage MIF with Alanine Inserted Between Pro-1 And Met-2. *Homo sapiens*	5542179	Lubetsky *et al.*, (1999).
Chain B, Macrophage MIF With Alanine Inserted Between Pro-1 And Met-2.Met-2. *Homo sapiens*	5542178	Lubetsky *et al.*, (1999).
Chain A, Macrophage MIF with Alanine Inserted Between Pro-1 and Met-2. *Homo sapiens*	5542177	Lubetsky *et al.*, (1999).
Macrophage migration inhibitory factor-like protein. *Trichuris trichiura*	CAB46355	
Macrophage MIF. *Bos taurus*	AAD38354	
Macrophage MIF. *Wuchereria bancrofti*	AAC82615	
Chain C, Macrophage MIF. *Homo sapiens*	1942979	Sun *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 93: 5191-6 (1996)
Chain B, Macrophage MIF. *Homo sapiens*	1942978	Sun *et al.*, (1996).
Chain A, Macrophage MIF. *Homo sapiens*	1942977	Sun *et al.*, (1996).
Macrophage MIF. *Meriones unguiculatus*	AAC02629	
Macrophage MIF. *Brugia*	AAB60943	

Name	GenPept Accession No.	Publication or Deposit
malayi		
Macrophage MIF. Bovine	S32394	Galat *et al.*, *FEBS Lett.* 319: 233-6 (1993).
Macrophage migration inhibitory factor DER6 – mouse.	A44499	Lanahan *et al.*, *Mol. Cell. Biol.* 12: 3919-29 (1992); Wistow *et al.*, (1993); Bernhagen *et al.*, (1993); Mikayama *et al.*, (1993); and Mitchell *et al.*, *J. Immunol.* 154: 3863-70 (1995).
Macrophage inhibitory factor (F5 cells) – human (fragment).	A61386	Oki *et al.*, *Lymphokine Cytokine Res.* 10: 273-80 (1991).
Macrophage migration inhibitory factor. *Homo sapiens*	CAA80598	Bernhagen *et al.*, (1993); and Wistow *et al.*, (1993).
MIF (rat liver), 115 aa	AAB32392	Sakai *et al.*, *Biochem. Mol. Biol. Int.* 33: 439-46 (1994).
p12a isoform = macrophage migration-inhibitory factor [cattle, Peptide, 114 aa].	AAB32021	Galat *et al.*, (1994).
Macrophage MIF {N-terminal partial peptide, 39 aa} *Bos taurus*.	AAB26003	Galat *et al.*, (1993).
Macrophage MIF. *Rattus norvegicus*	AAB04024	
Macrophage MIF. *Mus musculus*	CAA80583	Bernhagen *et al.*, (1993).
Macrophage MIF. *Mus musculus*	AAA91638	Kozak *et al.*, (1995).
Macrophage migration inihibitory factor. *Mus musculus*	AAA91637	Bozza *et al.*, *Genomics* 27: 412-19 (1995).
MIF. *Mus musculus*	AAA74321	Mitchell *et al.*, *J. Immunol.* 154: 3863-7 (1995).
Macrophage MIF. *Gallus gallus*	AAA48939	Wistow *et al.*, (1993).
Macrophage MIF. *Homo sapiens*	AAA36179	Wistow *et al.*, (1993).

Name	GenPept Accession No.	Publication or Deposit
Macrophage MIF. *Homo sapiens*	AAA21814	Paralkar *et al.*, (1994)
Macrophage MIF.-3 (human)		U.S. Patent Nos. 5,986,060; 5,650,295; ATCC No. 75712
Macrophage MIF.-2		Hirose *et al., Microbiol. Immunol.* 35:235-45 (1991).
Sequence 8 from U.S. Patent No. 5,807,714 (antigen-specific glycosylation inhibiting factor (AgGIF))	g5960276	U.S. Patent No. 5,897,714
Sequence 4 from U.S. Patent No. 5,807,714 (antigen-specific glycosylation inhibiting factor (AgGIF))	g5960275	U.S. Patent No. 5,807,714
Sequence 4 from U.S. Patent No. 5,807,714 (antigen-specific glycosylation inhibiting factor (AgGIF))	g5960274	U.S. Patent No. 5,897,714
Sequence 4 from U.S. Patent No. 5,807,714 (antigen-specific glycosylation inhibiting factor (AgGIF))	g5960273	U.S. Patent No. 5,897,714
Chain A, Human glycosylation-inhibiting factor	g1942169	Kato *et al.*, (1996).
Chain B, Human glycosylation-inhibiting factor	g1942170	Kato *et al.*, (1996).
Chain C, Human glycosylation-inhibiting factor	g1942171	Kato *et al.*, (1996).
Glycosylation-inhibiting factor - human	g2135300	Weiser *et al.*, (1989); and Paralkar *et al.*, (1994).
Glycosylation-inhibiting factor - bovine	g1085446	Galat *et al.*, (1994).
Glycosylation-inhibiting factor	g402717	Mikayama *et al.*, (1993).
Glycosylation-inhibiting factor	g402702	Mikayama *et al.*, (1993).

G. Method of Preparing Cell Lines Which Express Anti-MIF Antibodies

Once antibody secreting cells, which produce antibodies of a desired anti-MIF affinity, are isolated, these cells can be utilized using standard procedures to produce cell lines which produce the desired antibodies.

i. Hybridoma Preparation

Hybridomas secreting monoclonal antibodies can be prepared as described by Kohler and Milstein, *Nature* 256: 495-7 (1975) or by Galfré *et al., Methods Enzymol.* 73 (Pt. B): 3-46 (1981). Briefly, homozygous deficient MIF mice ($MIF^{-/-}$) are immunized by subcutaneous injection of about 0.1 to 100 :g (preferably 10 :g) of MIF protein in complete Freund's adjuvant, followed approximately 2 weeks later by intraperitoneal injection of about 10 :g of MIF in incomplete Freund's adjuvant. Antisera is collected about 1 week later and is analyzed in a micro-ELISA using microtiter plates coated with MIF protein (about 1 μ/ml) and detection of bound immunoglobulins with horseradish peroxidase-conjugated rabbit anti-mouse IgG. The specific antibody concentration in these antisera is retrospectively calculated by ELISA on microtiter plates coated with the respective antigen using purified monoclonal antibodies for calibration. After an interval of at least 4 weeks, the mice are boosted intraperitoneally with 10 :g of MIF protein in saline on days 4 and 2 before the cell fusion. Spleen cells are isolated and fused with either P3x63.Ag.8-6.5.3 or Sp2/O-AGl4 myeloma cells. After selection in hypoxanthine-aminopterine-thyinidine medium, the supernatants are screened for specific antibody production with an one-site, non-competitive, micro-ELISA using microtiter plates coated with MIF and detection of bound immunoglobulins as described above. Positive clones are used for the production of ascites in pristane-primed mice. The IgG fraction of the monoclonal antibodies can be purified from ascites by affinity-chromatography on protein A-Sepharaose.

It should be noted that injection schedules, the animal immunized, and the amount and type of MIF antigen used *(e.g.*, MIF fusion protein, MIF peptides or porteins) can be varied as would be known to the skilled artisan. See, *e.g.*, ED HARLOW *ET AL.*, ANTIBODIES: A LABORATORY MANUAL (1988).

ii. Antigen

The MIF antigen used to immunize the knock-out mice or other knock-out animal can be derived from various sources. MIF can be purified from biological samples by chromatography or other purification procedure. Alternatively, MIF can be prepared recombinantly in eukaryotes or prokaryotes as previously described. Whole MIF proteins can be injected into the animal, as well as MIF peptides. MIF peptides for use in raising anti-peptide anti-MIF antibodies are preferably greater than 6 consecutive MIF amino acids in length. Peptide antigens can be 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45 and 50 amino acids in length. Peptides can be prepared synthetically, recombinantly or by proteolytic cleavage of the MIF protein to produce proteolytic MIF fragments. Recombinant forms of MIF or MIF peptides can be in the form of a fusion protein, wherein MIF is fused to another protein or polypeptide such as maltose binding protein (MBP), Ǝ-galactosidase or other suitable protein. MIF peptides can also be expressed recombinantly.

H. *Diseases to be Treated Using Anti-MIF Antibodies*

Diseases mediated by MIF include inflammatory diseases, retinopathy, *e.g.* diabetic or SLE-associated retinopathy, delayed type hypersensitivity (DTH), conditions mediated by DTH, cancer, pathological conditions induced by viruses and other pathogens, adult respiratory distress syndrome (ARDS), autoimmune diseases, endotoxic shock, pathological conditions involving neovascularization and trauma.

In the instance of septic shock, MIF has been reported to be a major secreted protein released by anterior pituitary cells in response to lipopolysaccharide (LPS) and may be a critical mediator of septic shock (Calandra *et al., Nature* 377: 68-71 (1995); and Bernhagen *et al., Nature 365:* 756-9 (1993). Some have suggested that the counteraction or neutralization of MIF may serve as an adjunct therapy in sepsis (Bozza *et al., J. Exp. Med.* 189: 341-6 (1999)).

In cancer, MIF has been reported to be spontaneous expressed by human cancer cells (Shimizu *et al., Biochem. Biophys. Res. Commun.* 264: 751-8 (1999); and Bini *et al., Electrophoresis* 18: 2832-41 (1997)). MIF reportedly also mediates or is produced in elevated quantities in colonic adenomas (Shkolnik *et al., Am. J. Gastroenterol.* 82: 1275-8 (1987)), human T-cell leukemia virus (HTLV) induced T-cell leukemia (Koeffler *et al., Blood* 64: 482-90 (1984)), prostatic adenocarcinoma

(Meyer-Siegler *et al., Urology* 48: 448-52 (1996)), pseudolymphoma, sacroidosis, and acute myeloblastic leukemia (AML). Hypoxia can also induce transcription of MIF and MIF found, in the serum of head and neck cancer patients, has been correlated with the degree of hypoxia occurring in these patients (Koong *et al.*, *Cancer Res.* 60: 883-7 (2000)). MIF has been reported to suppress p53 activity and has been suggested as a link between inflammation and tumorigenesis (Hudson *et al.*, *J. Exp. Med.* 190:1375-82(1999)). Anti-MIF antibodies have been shown to inhibit growth and visualization of colon tumors in mice (Ogawa, 1999).

Delayed type hypersensitivity (DTH) related diseases include atopic dermatitis (Shimizu *et al., Biochem. Biophys. Res. Commun.* 240: 173-8 (1997)). Autoimmune diseases with potential MIF involvement include Gaucher's Disease, rheumatoid arthritis (see Leech *et al., Arthritis Rheum.* 42: 1601-8 (1999); Onodera *et al., J. Biol. Chem.* 275: 444-50 (2000); and Onodera *et al., Cytokine* 11: 163-7 (1999)), asthma, immunologically induced kidney disease and systemic lupus erythematosus. In rheumatoid arthritis, MIF seems to act by inducing expression of matrix metalloproteinases (MMPs), such as MMP-1 and MMP-3, by synoviocyte fibroblasts (Onodera *et al.*, 2000). MIF also has been indicated to play a role in psoriasis (Steinhoff *et al., Br. J. Dermatol.* 141: 1061-6 (1999)). Moreover, although it was known that MIF played a role in experimental glomerulonephritis (GN), only recently have researchers reported that MIF is markedly up-regulated in proliferative forms of human GN and that this up-regulation correlated with leukocyte infiltration, histologic damage and renal function impairment (Lan *et al., Kidney Int.* 57: 499-509 (2000)).

In one aspect, the anti-MIF antibodies or the immunogenic fragments thereof are contemplated for use in modulating the diseases and conditions described above. Preferably, the antibodies or their immunogenic fragments would inhibit the activity of MIF in a subject, wherein the subject is preferably human. More specifically, the anti-MIF antibodies contemplated are proposed for use alone or as an adjunct therapy to prevent disease progression. Some anti-MIF antibodies, prepared by methods other than those disclosed herein and with different specificities and affinities, have been shown to, for example, protect mice against (1) LPS-induced septic shock related death (Bernhagen *et al.*, 1993)); (2) lethal peritonitis induced by cecal ligation and puncture (CLP) (Calandra *et al., Nature Med.* 6: 164-70 (2000)), anti-glomerular basement membrane (GBM) induced glomerulonephritis (Lan *et al., J. Exp. Med.*

185: 1455-65 (1997)), collagen type II induced rheumatoid arthritis in mice (Mikulowska *et al., J. Immunol.* 158: 5514-7 (1997)) and adjuvant induced arthritis in rats (Leech *et al., Arthritis Rheum.* 41: 910-7 (1998)), and has slowed 38C13 B cell lymphoma growth and vascularization in mice (Chesney *et al., Mol. Med.* 5: 1181-91(1999)), and carcinoma growth and neovascularization (Ogawa *et al., Cytokine* 12: 309-14 (2000)).

I. Anti-MIF Antibody or Antibody Fragment Compositions and Administration

An antibody or fragment thereof of the invention is administered to subjects in a biologically compatible form suitable for pharmaceutical administration *in vivo*. By "biologically compatible form suitable for administration *in vivo*" is meant a form of the antibody or fragment to be administered in which any toxic effects are outweighed by the therapeutic effects of the antibody or fragment. An antibody or fragment can be administered in any pharmacological form, optionally in a pharmaceutically acceptable carrier. Administration of a therapeutically effective amount of the antibody or fragment thereof is defined as an amount effective, at dosages and for periods of time necessary to achieve the desired result *(e.g.*, inhibition of the progression or proliferation of the disease being treated). For example, a therapeutically active amount of an antibody or fragment thereof may vary according to such factors as the disease stage *(e.g.*, stage I versus stage IV), age, sex, medical complications, and weight of the individual, and the ability of the antibody or fragment thereof to elicit a desired response in the individual. The dosage regimen may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily, or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

The active compound, an antibody or fragment thereof, by itself or in combination with other active agents, such as conventional anti-cancer drugs, steroids *(e.g.*, glucocorticoids and cortico steroids) and additional antibodies or fragments thereof. Examples of steroids for use in combination with anti-MIF antibodies include dexamethasone and cortisol. Examples of glucocorticoids include: 21-Acetoxypregnenolone, Alclometasone, Algestone, Amcinonide, Beclomethasone, Betamethasone, Budesonide, Chloroprednisone, Clobetasol, Clobetasone,

Clocortolone, Cloprednol, Corticosterone, Cortisone, Cortivazol, Deflazacort, Desonide, Desoximetasone, Dexamethasone, Diflorasone, Diflucortolone, Difluprednate, Enoxolone, Fluazacort, Flucloronide, Flumethasone, Flunisolide, Flucinolone Acetonide, Fluocinonide, Fluocortin Butyl, Fluocortolone, Fluorometholone, Fluperolone Acetate, Fluprednidene Acetate, Fluprednisolone, Flurandrenolide, Fluticasone Propionate, Formocortal, Halcinonide, Halobetasol Propionate, Halometasone, Halopredone Acetate, Hydrocortamate, Hydrocortisone, Loteprednol Etabonate, Mazipredone, Medrysone, Meprednisone, Methylprednisolone, Mometasone Furoate, Paramethasone, Prednicarbate, Prednisolone, Prednisolone 25-Diethylaminoacetate, Prednisolone Sodium Phosphate, Prednisone, Prednival, Prednylidene, Rimexolone, Tixocortol, Triamcinolone, Triamcinolone, Acetonide, Triamcinolone Benetonide, Triamcinolone Hexacetonide. The immunoconjugate, alone or in combination with other agents, may be administered in a convenient manner such as by injection (subcutaneous, intramuscularly, intravenous, *etc.),* inhalation, transdermal application or rectal administration. Depending on the route of administration, the active compound may be coated with a material to protect the active compound from the action of enzymes, acids and other natural conditions, which may inactivate the compound. A preferred route of administration is by intravenous (I.V.) injection. Examples of conventional anti-cancer drugs include, but are not limited to methotrexate, taxol, cisplatin, tamoxifen, et seq.

To administer an antibody or fragment thereof by other than parenteral administration, it may be necessary to coat the antibody or fragment thereof with, or co-administer the antibody or fragment thereof with, a material to prevent its inactivation. For example, an antibody or fragment thereof can be administered to an individual in an appropriate carrier or diluent, co-administered with enzyme inhibitors or in an appropriate carrier or vector, such as a liposome. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Liposomes include water-in-oil-in-water emulsions, as well as conventional liposomes (Strejan *et al., J. Neuroimmunol.* 7: 27 (1984)). Additional pharmaceutically acceptable carriers and excipients are known in the art or as described in REMINGTOM'S PHARMACEUTICAL SCIENCES (18th ed. 1990).

The active compound may also be administered parenterally or intraperitoneally. Dispersions of the active compound also can be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations may contain one or more preservatives to prevent the growth of microorganisms.

Pharmaceutical compositions suitable for injectable use include sterile, aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the composition must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion, and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorBio acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols, such as manitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating an active compound *(e.g.,* an anti-MIF antibody or fragment thereof) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle, which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying, which yields a powder of an active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

When the active compound is suitably protected, as described above, the protein may be orally administered, for example, with an inert diluent or an assimilable edible carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the therapeutic compositions is contemplated. All compositions discussed above for use with an anti-MIF antibody or fragment thereof may also comprise supplementary active compounds in the composition.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of a dosage. "Dosage unit form," as used herein, refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound is calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on: (A) the unique characteristics of the active compound and the particular therapeutic effect to be achieved; and (B) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

J. In Vitro *Functional Assays for Testing MIF-Neutralizing Antibodies*

Several assays are available for testing whether a particular anti-MIF antibody produced from a MIF knockout animal, or a humanized antibody a portion of which is derived from an anti-MIF antibody produced from a knock out animal neutralize MIF-induced activity.

For example, one assay is the phenylpyruvate tautomerase (PPT) assay. This assay is based on the fact that MIF interconverts the enol- and keto- forms of phenylpyruvate and (p-hydroxyphenyl)pyruvate (Hermanowski-Vostka *et al.*, *Biochem.* 38: 12841-9 (1999)). As shown in Fig. 3, MIF catalyzes the tautomerization of *p*-hydroxyphenylpyruvate. MIF also has been shown to possess D-dopachrome tautomerase and thiol protein oxidoreductase activities (Matsunaga *et al.*,

Cell. Mol. Biol. 45: 1035-40 (1999)). Accordingly, similar assays could be developed for the D-dopachrome and thiol protein oxidoreductase activities, as is described for the PPT assay.

Another *in vitro* assay can be performed in, for example NIH-3T3 cells, to determine the MIF activity inhibition based on the role of MIF (see Fig. 4) in the activation of the p44/p42 extracellular signal-regulated (ERK) mitogen-activated protein kinases (MAP) pathways, as discussed by Mitchell *et al., J. Biol. Chem.* 274: 18100-6 (1999). The activation of ELC by ERK ½ is discussed by Mitchell *et al.,* (1999). The assay is a transcription-based assay for testing the efficacy of MIF neutralization by anti-MIF antibodies. A construct comprising a serum response element (SRE), promoter and Secreted alkaline phosphatase (SEAP) is created and transiently transfected into an appropriate cell line, such as NIH-3T3 cells. The expression of the SEAP gene is proportional to the transcriptional activation of ELK1 (e.g., EL1 – pELK1^{+}). The impact of anti-MIF antibodies on MIF stimulated SRE-mediated transcription ascedrtained by measuring the alkaline phosphatase concentrations secreted (see Fig. 5). The alkaline phosphatase can be assessed using, for example a chemiluminescence detection system. Similar studies can be performed on MIF signaling events involving other phosphorylation of pathways involving transcription activation of AP-1, NF-KB and other factors.

Other *in vitro* studies for examining the activity of MIF signaling includes growth arrest and apoptosis studies. The potential target interactions include a MIF-mediated cascade involving override of 53 effects, tumor necrosis factor ∀ (TNF), sodium nitroprusside and glucocorticoids. *In vitro* assay systems, such as those described above, could be suitably altered to study each of these interactions, and thereby study the anti-MIF activity of the antibodies or fragments thereof in inhibiting said MIF activity.

Another assay would be based on MIF induction of MMP-l release from cells. As discussed, MIF can up-regulate the matrix metalloproteinases (MMPs), such as MMP-1 (interstitial collagenase) and MMP-3 (stromelysin) (Onodera *et al.,* 2000). Anti-MIF antibodies can then be tested for their ability to inhibit MIF induced MMP-l release, for example, from human adult dermal fibroblasts. Other cells which produce MMPs would also be suitable for such assays, such as MMP-l release from RA synovial fibroblasts.

Still another bioassay includes anti-MIF antibody inhibition of VEGF-stimulated endothelial cells. These assays include changes in proliferation and regulation of cell cycle and apoptosis.

K. In Vivo *Models for Testing MIF-Neutralizing Antibodies*

There are several *in vivo* models for testing the efficacy of a particular anti-MIF monoclonal antibody in an animal model. Lipopolysaccharide (LPS) induced disease is an animal model in which to examine septic shock (see, e.g., Bernhagen *et al., Nature* 365: 756-9 (1993)). Spontaneous mouse glomerulonephritis (GN) in mice strains such as female NZB/W Fl and NZM2410; GN can also be rapidly induced by injection of rabbit anti-GBM (glomerular basement membrane) serum (Lan *et al., J. Exp. Med.*. 185: 1455-65 (1997)). The animal models of adjuvant-induced arthritis (see Leech *et al., Arthritis Rheum.* 41: 910-7 (1998)) in rats, and collagen type II induced arthritis in mice (see Mikulowska *et al., J. Immunol.* 158: 5514-7 (1997)) are appropriate animal models for studying methods of treating human rheumatoid arthritis.

These animal models would be used to determine the inhibitory activity of anti-MIF monoclonal antibodies on MIF-induced activity in each of these diseases. For example, in the MIF/LPS lethality animal model, mice would be preinjected with an anti-MIF monoclonal antibody or negative control antibody. Two hours later the mice would receive an injection of MIF and LPS. Seven hours after the injection of MIF and LPS, the mice would receive an injection of MIF alone. The number of mice which survive this regimen of LPS-induced lethality would then be examined as compared to the control mice receiving an antibody other than an anti-MIF antibody (control) or mice not receiving any LPS. Survival would be plotted, typically at 24 hr, 48 hr, 72 hr and 96 hr after the MIF and LPS injection.

Uses

The present invention further is directed to use of anti-MIF antibodies for treatment and prophylaxsis of diseases, wherein suppression or modulation of MIF is therapeutically beneficial. Examples thereof include diseases involving cytokine-mediated toxicity. More specific examples are inflammatory diseases and autoimmune diseases, such as rheumatoid arthritis and other autoimmune diseases,

graft-vs-host disease, TNF induced toxicity, endotoxin associated toxicity, septic shock, infections such as malarial, bacterial and viral infections, allergy, etc. Also, anti-MIF antibodies can be used to suppress undesirable immune responses. Such antibodies may be administered alone or in combination with other active agents, as described above.

The examples and methods provided below serve merely to illustrate particular embodiments of the invention and are not meant to limit the invention.

EXAMPLE 1

Preparation of a MIF Knock-Out Mouse

Targeting vector construction and generation of MIF$^{-/-}$ Mice. A mouse *MIF* genomic fragment is isolated from a 129SV/J genomic library (Bozza *et al., Genomics* 27: 412-19 (1995)), and a 6.1 kb XbaI fragment containing the 5' upstream region, exons 1-3, and the 3' region is subcloned in pBluescript®. The vector is digested with EcoRV (sites present in the 3' region of the gene and in the polylinker of the plasmid), releasing a 0.7 kb fragment. The vector is religated and digested with AgeI, which disrupts part of exon 2, the second intron, and exon 3. The *neo* cassette is inserted by blunt ligation after end-filling the vector and the *neo* cassette. The disrupted genomic vector is digested with XbaI/XhoI and ligated into the HSV-TK vector. The targeting vector is linearized with XhoI, and 30 μg is transfected by electroporation into 10^7 Jl embryonic stem (ES) cells that are maintained on a feeder layer of *neo* embryonic fibroblasts in the presence of 500 U/ml of leukemia inhibitory factor. After 8 days of selection with G418 (200 μg/ml) and FIAU (2 μg), 30 clones are analyzed by Southern blot hybridization using the 0.7 kb EcoRV/XbaI 3' fragment as a probe. An ES cell line clone displaying a novel 7 kb XbaI allele predicted to occur after homologous recombination is injected into day 3.5 C57BL/6 blastocysts. The blastocysts are transferred into pseudopregnant females. Chimeric mice are bred with C57BL/6 mice and agouti offspring can be analyzed for the *MIF* disrupted allele by Southern blot hybridization.

Results. The mouse *MIF* gene can be disrupted by replacing part of exons 2 and 3 with a *neo* cassette. The targeting vector is electroporated in J1 ES cells and G418-FIAU-resistant colonies are isolated. Correctly targeted ES cells are used to

generate chimeric animals by injection into C57BL/6 blastocysts. Highly chimeric animals transmitted the mutated allele through the germline. Homozygous mice are generated by intercrosses of heterozygous mice. Northern blot analysis from liver RNA of lipopolysaccharide (LPS)-treated animals can be used to confirm that the gene disruption creates a null mutation (Bozza *et al., J. Exp. Med.* 189: 341-6 (1999)). ELISA of serum from LPS-treated animals can be used to further confirm the absence of MIF protein in the $MIF^{-/-}$ mice (see Bozza *et al.,* 1999). As described by Bozza *et al.*, of the 218 animals obtained from heterozygous matings described above, 16% were homozygous for the null allele. The newborn $MIF^{-/-}$ mice developed normally in size and behavior and were fertile. The litter size of heterozygous and homozygous matings were normal. Both gross examination and histopathological analysis of several organs (kidney, liver, spleen, adrenal, thymus, lungs, heart, brain and intestine) of $MIF^{-/-}$ mice revealed no abnormalities. Flow cytometric analysis of splenocytes and thymocytes of $MIF^{-/-}$ mice demonstrated normal lymphocyte populations (Bozza *et al.,* 1999).

EXAMPLE 2

Preparation of Anti-MIF Antibodies in a MIF Knock Out Mouse

Six week old mice, which are MIF knock out mice, are immunized by subcutaneous injection of 100 μg of MIF protein, MIF peptides fragment in Freund's Complete Adjuvant on day one, followed by a similar injection in Freund's Incomplete Adjuvant at day 10. Intraperitoneal injections are then performed at weekly intervals of 100 μg of MIF (or a MIF peptide fragment) in phosphate buffered saline (PBS). Blood is collected by supraorbital functions.

EXAMPLE 3

Preparation of Hybridomas

For hybridoma fusion, the spleen of the mice immunized in Example 2 are isolated and $1x10^8$ splenocytes are fused to an equal number of Ag8 myeloma cells using the standard polyethylene glycol protocol. Selection in hypoxanthine/aminopterine/ thymidine is initiated directly after replating the cell suspension into fifteen 96-well flat bottom plates. Supernatants are screened 10-14

days after the hybridoma fusion. Positive hybridomas can then be repeatedly subcloned.

Analysis of antibody affinity can be assayed by ELISA. For example, one μg of protein/ml PBS is coated in a 96-well polyvinylplate for 3 hours at 37°C. After three washes with PBS/0.05% Tween-20, the plates are blocked with PBS/0.1% bovine serum albumin (BSA) for 1 hour at 37°C. Again three washes are performed before the first antibody is incubated. Sera or antibodies are diluted in PBS/0.05% Tween-20/1% fetal calf serum (FCS). The serum incubation is performed for 1 hour at 37°C, followed by 3 washes. The enzyme conjugate RAMPO (Dakopats), is diluted 1000-fold and incubated for 1 h at 37°C. Tetra methyl benzidine (TMB) is used as the substrate for the peroxidase reaction. This reaction is stopped after 15 minutes, at room temperature by adding equal volume of 1 N H_2S0_4, at which time the optical density can be measured at 450 nm. As noted in Table 2 below, no high affinity anti-MIF generating hybridomas were produced from BALB/c mice, whereas using the MIF knockout mouse, numerous anti-MIF producing hybridomas were generated.

TABLE 2

Generation of Mabs that Bind MIF with High Affinity

mouse	immunogen	#fusions	fusion date	# hybridomas generated	#anti-MIF hybridomas
BALB/c	MIF	2	12-22-98	573	0
BALB/c	MIF	2	03-11-99	344	0
BALB/c	MIF	2	05-10-99	384	0
BALB/c	MIF	3	08-05-99	500	0
BALB/c	MIF/OVA	1	12-21-99	?	0
MIF KO	MIF/OVA	4	12-21-99	3242	671
MIF KO	MIF	3	02-14-00	2304	12

KO = MIF knockout mouse

EXAMPLE 4

Phenylpyruvate Tautomerase Assay

The assay for relative phenylpyruvate tautomerase activity of MIF was modified from Lubetsdy et al., *Biochemistry*, 38: 7346-7354 (1999). We used p-hydroxyphenylpyruvate (HPP) (Aldrich) as substrate. HPP was dissolved in 50 mM ammonium acetate (pH 6.0) at room temperature for overnight and stored in refrigerator until use. For catalytical activity measurement, 20 µl of HPP was added to 1.96 ml of 0.435 M boric acid (pH 6.2) and allowed to equilibrate in 1 ml quartz cuvette at room temperature for five minutes. To initiate the catalytic activity, 20 µl of 0.01 mg/ml rhMIF was added to above solution and thoroughly mixed. Activity was measured by following the increase in absorbance at 330 nm for five minutes. To study the effect of mouse anti-MIF anticlonal antibodies on the phenylpyruvate tautomerase activity of rhMIF, 0.2 µg of rhMIF is pre-incubation with 12.5 µg of antibody at 25°C for one hour, then the 30 µl protein mixture was added to 1.97 ml of assay solution that contains HPP. For each antibody clone, the mean activity (slope of absorbance increase) was calculated from triplicate measurements. The relative activity was calculated by taking the percentage for the slope of the antibody-rhMIF samples to that of the rhMIF alone.

As shown in Fig. 3, when anti-MIF monoclonal antibodies (12.5 μg monoclonal antibody) are added to the reaction mixture containing MIF (0.2 μg MIF), the antibodies inhibits PPT activity. These results are summarized in Table 3 below. The outcomes are presented in the percent MIF-induced PPT activity remaining after the addition of each anti-MIF antibody.

TABLE 3

Anti-MIF Mabs Effects on MIF Phenylpyruvate tautomerase activity

(12.5 Mg Mab + 0.2 μg MIF)

Antibody	Subelone Off-Rate	% MIF PPT Activity
30B7-11	<1.0E-06	**-12**
19B11-7	1.0E-05	**0**
22F11-6	2.0E-05	**65**
34D11-1	5.0E-05	**54**
2D8-3	8.0E-06	**57**

33G7-9	<1.0E-06	**67**
6B5-5	<1 .0E-06	**82**
2G2-5	6.0E-05	**92**
9G10-12	3.6E-05	**98**
2B8 (murine anti-CD2O Mab)	-	**105**
1OB11-3	9.0E-06	**136**
IA9-7	2.0E-05	**136**
29B12-1	<1.0E-06	**146**
IIA9-8	<1.0E-06	**159**
6E2-12	<1.0E-06	**188**

EXAMPLE 5

Anti-MIF Mab Inhibition of MIF Induced SRE-SEAP Transcription/Secretion

The results depicted in Fig. 6 demonstrate that the addition of anti-MIF antibodies to a reaction containing MIF inhibited the stimulation of MIF induced SRE-SEAP transcription and secretion. The most inhibitory of the antibodies tested were the 6B5-5, 2G2-5 and 22F11-6 antibodies.

EXAMPLE 6

MIF Stimulated MMP- 1 Release Assay

MIF is known to stimulate MMP-1 release from normal synovial fibroblasts or rheumatoid arthritis [Onodera, et al. (2000)]. When anti-MIF antibodies are added along with MIF, MIF stimulated MMP-l release from the fibroblasts is inhibited (Fig. 7). The antibodies l0B11-3, 2D8-3, 19B11-7 and 33G7-9 all inhibit MIF-induced MMP-1 release (Fig. 7, upper left panel). Additionally, 22F11-6, 6B5-5, 34D11-1, 9G10-12 and 2G2-5 also inhibit MIF induced MMP-l release (Fig. 7, upper right panel). Of these, the antibodies which prevent MIF-induced MMP-1 release, according to Fig. 7, lower panel, were l0B11-3, 6B5-5 and 22F11-6. Additional antibodies were tested for MIF inhibitory activity of MMP-1 release as seen in Fig. 8. In all instances, the concentration of antibody administered was 10 μg/m1. The results from this Example and Example 5 above can be summarized in Table 4 below:

TABLE 4

Anti-MIF Monoclonal Antibody Effects on In Vitro *Bioassays*

MAB	Subclone Off-Rate	%MIF PPT Activity	10 µg mab/ml MIF Stimulated SRE Transcription (%MIF Activity)	10 µg mab/ml MIF Stimulated MMP-1 Release (% MIF Activity)	Binds Human MIF	Binds Murine MIF
None	-	-	100%	100%	N/A	N/A
10B11-3	9.0E-06	136	0 (-83%)	0 (-14.2%)	<0.1E-09 Kd	-
22F11-6	2.0E-05	65	42%	0 (-4.3%)	<0.1E-09 Kd	+
6B5-5	<1.0E-06	82	36%	0 (-2.5%)	++	-
2D8-3	8.0E-06	57	0 (-21%)	+		
34D11-1	5.0E-05	54	0%	+		
33G7-9	<1.0E-06	67	5%	+	++	++
29B12-1	<1.0E-06	146	25%	+	++	-
19B11-7	1.0E-05	0	29%	+	<0.1E-09 Kd	+++
2G2-5	6.0E-05	92	32%	+	++	-
6E2-12	<1.0E-06	188	0 (-250%)	-		
30B7-11	<1.0E-06	-12	0 (-53%)	-		
IA9-7	2.0E-05	136	0 (-46%)	-		
9G10-12	3.6E-05	98	0 (-22%)	-		
IIA9-8	1.0E-06	159	122%	-		
Mab					Kd	
24-31 (murine anti-CD154 mab)		-		-		
IDEC-114 (anti-CD80 mab)					2.2E-09	
IDEC-152 (anti-BD23 mab)					1.2E-09	

Also supplied in Table 4 is the human MIF and murine MIF binding capabilities of each of the listed antibodies.

5

EXAMPLE 7

MIF/LPS Lethality Model For Assessing Anti-MIF Antibodies

BALB/c mice were injected (all injections i.p. in this experiment) with lipopolysaccharide (LPS strain: E cell 0111:BY, Sigma Catalog #L2630) at 10 mg LPS/kg body weight. Some of the LPS-treated mice were then injected with 5 mg/kg monoclonal antibody (negative control) or an anti-MIF antibody (specific to human MIF). Additionally, MIF (R&R MIF Lot #US1600MBCO) was administered to said mice at a concentration of 2.5 mg/kg at the time of LPS injection (T=0) and seven hours later (T=7 hours). Mice pre-treated with anti-MIF monoclonal antibody at T=-2 hours had a greater percent survival than animals which received LPS and MIF or LPS and MIF and the negative-control antibody. These results are in Fig. 9.

A similar experiment was conducted wherein BALB/c mice were treated as described above, except that 12.5 mg/kg of LPS was administered instead of 10 mg/kg. As shown in Fig. 10, mice pre-treated with anti-MIF again had greater survival percentage than animals which did not receive antibody or which received the negative-control antibody.

Further, another similar experiment was effected except that 15.0 mg/kg LPS body weight was administered (rather than the previous 10.0 or 12.5 mg/kg body weight). Again, the animals which received anti-MIF had better survival percentages than animalsl which did not receive antibody or received the negative-control antibody.

These results are summarized in Table 5 as well as other activities of the tested antibodies specific to MIF.

TABLE 5

Anti-MIF Mab Effects on MIF + LPS Lethality in BALB/c Mice

MAB	%MIF PPt Activity	10 µg mab/ml MIF Stimulated SRE Transcription (%MIF Activity)	10 µg mab/ml MIF Stimulated MMP-1 Release (% MIF Activity)	Binds Human MIF	Binds Murine MIF	Blocks LPS Lethality
10B11-3	136	0 (-83%)	0 (-14.2%)	<0.1E-09 Kd	-	
22F11-6	65	42%	0 (-4.3%)	<0.1E-09 Kd	+	+

6B5-5	82	36%	0 (-2.5%)	++	-	
2D8-3	57	0 (-21%)	+			
34D11-1	54	0%	+			
33G7-9	67	5%	+	++	++	+
29B12-1	146	25%	+	++	-	+
19B11-7	0	29%	+	<0.1E-09 Kd	+++	+
2G2-5	92	32%	+	++	-	
6E2-12	188	0 (-250%)	-			
30B7-11	-12	0 (-53%)	-			
IA9-7	136	0 (-46%)	-			
9G10-12	98	0 (-22%)	-			
IIA9-8	159	122%	-			
24-31 (murine anti-CD154 mab)			-			-

The characteristics of two of the lead candidate antibodies are summarized below in Table 6:

TABLE 6

	Mab 10B11-3	Mab 22F11-6
human MIF Kd<50nm	<0.1nM	<0.1nM
Neutralizes MIF *in vitro* 10 µg/m1 MIF stimulated transcription SRE:. SEAP	100%	58%
Neutralizes MIF *in vitro* 10 µg/ml MIF stimulated MMP-1 release	100%	100%

The following Table 7 lists the antibodies generated from MIF knockout (KO) mice as well as anti-CD80 and anti-CD23 antibodies.

TABLE 7

Summary of Anti-MIF mabs generated from MIF gene knockout mice					
		Parent CGM	Parent CGM	Subclone CGM	
Hybridoma	Fusion	Off-Rate #1	Off-Rate #2	Off-Rate	HYBRIDOMA STATUS
29B12-1	1	3.5E-05	<1.0E-06	<1.0E-06	purified from ascites fluid
30B7-11	1	9.4E-06	<6.0E-06	<1.0E-06	purified from ascites fluid
IIA9-8	1	3.6E-05	<6.0E-06	<1.0E-06	purified from ascites fluid
6B5-5	1	1.7E-05	3.0E-05	<1.0E-06	purified from ascites fluid
33G7-9	1	1.8E-05	-	<1.0E-06	purified from ascites fluid
6E2-12	1	2.9E-05	6.0E-05	<1.0E-06	purified from ascites fluid
2D8-3	1	8.8E-05	4.5E-05	8.0E-06	purified from ascites fluid
10B11-3	1	1.7E-05	<1.0E-06	9.0-E06	purified from ascites fluid
19B11-7	1	3.4E-06	<1.0E-06	1.0E-06	purified from ascites fluid
L2E1-9	1	1.0E-05	<1.0E-05	1.0E-06	expanded for CGM
IA9-7	1	4.5E-05	<1.0E-06	2.0E-05	purified from ascites fluid
22F11-6	1	6.6E-05	2.0E-05	2.0E-05	purified from ascites fluid
7E10-11	1	9.4E-05	8.0E-05	2.0E-05	expanded for CGM
25D11	1	3.0E-05			purified from CGM
25D-11		2.8E-05			subclones frozen
9G10-12	1	2.8E-05	<1.0E-06	3.6E-05	purified from ascites fluid
22A5-12	1	9.2E-05	5.0E-05	4.0E-05	expanded for CGM

14H5	1	4.0E-05			subclones frozen
34D11-1	1	7.1E-05	1.0E-05	5.0E-05	purified from ascites fluid
IDEC-114 (anti-CD80 mab)				**5.4E-05**	
2G2-5	1	9.4E-05	4.0E-5	6.0E-05	purified from ascites fluid

Summary of Anti-MIF mabs generated from MIF gene knockout mice					
		Parent CGM	Parent CGM	Subclone CGM	
Hybridoma	Fusion	Off-Rate #1	Off-Rate #2	Off-Rate	HYBRIDOMA STATUS
L3A11-5	2	1.0E-05	8.0E-05	7.0E-05	expanded for CGM
L4A10-8	2	1.0E-05	5.0E-05	8.0E-05	expanded for CGM
K8H8-9	2	1.0E-05	<1.0E-05	9.0-E-05	expanded for CGM
33C4	1	9.4E-05			subclones frozen
L4C9-4	2	2.0E-04	8.0E-05	1.0E-05	purified from CGM
K8C9-8	2	2.0E-04	1.0E-05	1.0E-05	expanded for CGM
L1A6-7	2	3.0E-04	N/D	1.0E-05	purified from CGM
22C11-8	1	2.0E-04	1.6E-04	1.6E-04	ready to purify from CGM
IIB1-4	1	2.2E-05	1.0E-05	2.0E-04	expanded for CGM
11H2-9	1	8.1E-05	1.5E-04	2.0E-04	expanded for CGM
33F6-10	1	7.8E-05	2.0E-04	2.0E-04	expanded for CGM
19D3-9	1	6.3E-05	3.0E-04	2.0E-04	purified from CGM
L4G3	2	2.0E-04			subclones frozen
5A11-10	1	8.9E-05	3.0E-05	4.0E-05	expanded for CGM
IDEC-152 (anti-CD23 mab)				**4.8E-04**	
Total monoclonal hybridomas					**34**
Total mabs purified from ascites fluid					**14**
Total mabs to be purified from CGM					**20**

EXAMPLE 8

Identification of Sequences

The DNA and amino acid sequences of several lead candidate antibodies were identified, particularly for 6B5, 10B11, 19B11, 22F11, 29B12 and 33G7 and are contained in Figs. 17-30. These sequences may be further mutated in order to enhance binding affinity.

EXAMPLE 9

Administration of an Anti-MIF Antibody for Therapy

Anti-MIF antibody is administered at doses that may range from 1-5 mg/kg to patients with an inflammatory disease who are not being treated with other drugs, or to those who are being treated with steroids such as Dexamethasone or other anti-inflammatory drugs. In certain cases of chronic inflammatory conditions such as asthma, RA or nephritis, the combination treatment with anti-MIF antibody and, for example, steroids may lead to the reduction of the steroid maintenance dose. Under such conditions the antibody may be used as a steroid salvage therapy which will bring the steroid dose down to avoid the side effects of steroid high dose therapy. The anti-MIF antibody may be administered i.v., i.m. or s.c. at intervals that may vary from weekly to monthly dosing regimens.

Although the present invention has been described in detail with reference to examples above, it is understood that various modifications can be made without departing from the spirit of the invention. All references discussed above are hereby incorporated by reference in their entirety.

CLAIMS

What is claimed is:

1. A method of preparing a high-affinity anti-MIF antibody or fragment thereof comprising the steps of:

(a) preparing a transgenic animal in which the *MIF* gene is functionally knocked out;

(b) immunizing said transgenic animal with a MIF protein or a polypeptide fragment thereof; and

(c) obtaining a high-affinity anti-MIF antibodies or fragment thereof from said animal.

2. The method of Claim 1, wherein the high-affinity anti-MIF antibody or fragment thereof recognizes and binds to MIF- 1 or fragment thereof, MIF-2 or fragment thereof, MIF-3 or fragment thereof, or a MIF-like protein or fragment thereof.

3. The method of Claim 1, wherein the animal is selected from the groups consisting of rodent, canine, porcine, feline, equine, ovine and bovine.

4. The method of Claim 3, wherein the rodent is selected from the groups consisting of rat, mouse, hamster and guinea pig.

5. The method of Claim 1, wherein the MIF or immunogenic polypeptide thereof is selected from human MIF-1, human MIF-2, human MIF-3, a MIF fusion protein, or a MIF peptide of at least about 7 consecutive amino acids.

6. The method of Claim 1, wherein the high-affinity anti-MIF antibody or fragment thereof is an anti-peptide antibody specific to a MIF epitope, a humanized antibody, a human antibody or a chimeric antibody.

7. A high-affinity anti-MIF antibody produced by the method of Claim 1.

8. A high-affinity anti-MIF antibody produced from a MIF knockout mouse, wherein said antibody is characterized by: (1) binding soluble human MIF with an affinity of ≤ 50 nM; or (2) blocking MIF-induced activity at a concentration of 10 μ/ml or less.

9. The high affinity antibody of claim 8, wherein the MIF-induced activity which is blocked is one or more of the following: MIF stimulated MMP release, PPT activity, LPS lethality, or MIF stimulated SRE transcription.

10. The high affinity antibody of claim 9, wherein the MMP stimulated by MIF is selected from MMP- 1 and MMP-3.

11. The high-affinity anti-MIF antibody of claim 8, wherein said antibody binds soluble human MIF with an affinity of ≤ 0.1 nM; or (2) blocking MIF-induced activity at a concentration of 10 μ/ml or less.

12. A high affinity anti-MIF monoclonal antibody produced from a knock-out mouse, wherein the monoclonal antibody is 10B11-3, 22F11-6, 6B5-5, 29B12-1, 19B11-7 or 33G7-9.

13. A nucleic acid encoding an anti-MIF monoclonal antibody of claim 12.

14. A vector comprising the nucleic acid of claim 13.

15. An isolated cell transfected with the vector of claim 14.

16. A high affinity anti-MIF monoclonal antibody produced from a knock-out mouse, wherein the monoclonal antibody is lOB11-3, 22F11-6, 29B12-1, 30B7-11, 11A9-8, 6B5-5, 33G7-9, 6E2-12, 2D8-3, l0B11-3, 19B11- 7, L2El-9, 1A9-7, 22F11-6, 7E10-11, 25D-11, 9G10-12, 22A5-12, 14H5 and 34D11-1.

17. A nucleic acid encoding an anti-MIF monoclonal antibody of claim 16.

18. A high affinity, humanized anti-MIF antibody, wherein said antibody is characterized by having at least one of the following: (1) binds soluble human MIF with an affinity of ≤ 50 nM; or (2) blocks MIF-induced activity *in vitro* at concentrations of 10 μ/ml or less.

19. The high affinity, humanized anti-MIF antibody of claim 18, wherein the antibody is also cross reactive with murine MIF.

20. The high-affinity anti-MIF antibody of Claim 7, wherein the antibody recognizes and binds to MIF-1 or fragment thereof, MIF-2 or fragment thereof, MIF-3 or fragment thereof or a MIF-like protein or fragment thereof.

21. A method of preparing a cell line producing a high-affinity monoclonal anti-MIF antibody or fragment thereof by preparing hybridomas using the cells producing the anti-MIF antibodies of Claim 1.

22. The method of Claim 21, wherein the high-affinity anti-MIF antibody or fragment thereof recognizes and binds to MIF-1 or fragment thereof, MIF-2 or fragment thereof, MIF-3 or fragment thereof, or a MIF-like protein or fragment thereof.

23. A high-affinity anti-MIF monoclonal antibody or fragment thereof produced by the cell line of Claim 21.

24. The high affinity anti-MIF antibody fragment of Claim 23, wherein the fragment is selected from the group consisting of: FV, scFV, Fab, Fab' and $F(ab')_2$.

25. The high-affinity anti-MIF monoclonal antibody or fragment thereof of Claim 6, wherein the antibody has a dissociation constant (K_D) of about 10^{-8} M to about 10^{-9} M or less for a MIF epitope.

26. An isolated nucleic acid comprising a MIF targeting construct comprising (A) a selectable marker and (B) DNA sequence homologous to a *MIF* gene, wherein said isolated nucleic acid is introduced into an animal at an embryonic stage, and wherein said nucleic acid disrupts endogenous *MIF* gene activity wherein MIF protein production is blocked and wherein said animal is suitable for production of high-affinity anti-MIF antibodies.

27. The isolated nucleic acid of Claim 26, wherein the MIF targeting construct targets a *MIF-1* gene, a *MIF-2* gene, a *MIF-3* gene or a gene encoding a MIF-like protein.

28. The isolated nucleic acid of Claim 26, wherein the selectable marker sequence confers a positive selection characteristic.

29. The isolated nucleic acid of Claim 26, wherein the selectable marker is a neomycin resistance *(neo)* gene.

30. A transgenic animal genome comprising a homozygous disruption of the endogenous *MIF* gene *($MIF^{-/-}$),* wherein said disruption comprises the insertion of a selectable marker sequence, and wherein said disruption results in said animal with negligible or no expression of MIF as compared to a wild type animal and wherein said animal is capable of producing high affinity anti-MIF antibodies.

31. The transgenic animal of Claim 30, wherin the *MIF* gene which is homozygously disrupted is selected from the group consisting of a *MIF-1* gene, a *MIF-2* gene, a *MIF-3* gene or gene encoding a MIF-like protein.

32. The transgenic animal of Claim 30, wherein the selectable marker sequence is a neomycin cassette.

33. The transgenic animal of Claim 30, wherein the anti-MIF antibodies produced by said animal have a dissociation constant (KD) for a MIF epitope of about l0 M to about 10-9 M or less.

34. A method for producing a transgenic animal lacking an endogenous *MIF* gene, said method comprising:

(a) introducing a MIF targeting construct comprising a selectable marker sequence into an embryonic stem (ES) cell or ES-like cell;

(b) introducing said animal ES cell or ES-like cell into an animal embryo;

(c) transplanting said embryo into a pseudopregnant animal;

(d) allowing said embryo to develop to term; and

(e) identifying a transgenic animal whose genome comprises a disruption of the endogenous *MIF* gene at least one allele;

(f) breeding the transgenic animal of step E to obtain a transgenic animal whose genome comprises a homozygous disruption of the endogenous *MIF* gene *($MIF^{-/-}$)*, wherein said disruption results in an animal which lacks endogenous MIF as compared to a wild type animal.

35. The method of Claim 34, wherein the embryonic stem cell is a mouse embryonic stem cell and the animal is a mouse.

36. A nucleic acid encoding a high-affinity anti-MIF monoclonal antibody or fragment thereof of Claim 23.

37. A therapeutic composition comprising an anti-MIF antibody or fragment thereof of Claim 23 and a pharmaceutically acceptable carrier.

38. The therapeutic composition of Claim 37 further comprising a steroid.

39. The therapeutic composition of claim 37 further comprising an immunosuppressive, cytotoxic or other anti-cancer agent.

40. The therapeutic composition of Claim 38, wherin the steroid is a glucocorticoid or a corticosteroid.

41. The therapeutic composition of Claim 40, wherein the glucocorticoid is selected from the group consisting of: 21-Acetoxypregnenolone, Alclometasone, Algestone, Aincinonide, Beclomethasone, Betamethasone, Budesonide, Chloroprednisone, Clobetasol, Clobetasone, Clocortolone, Cloprednol, Corticosterone, Cortisone, Cortivazol, Deflazacort, Desonide, Desoximetasone, Dexamethasone, Diflorasone, Diflucortolone, Difluprednate, Enoxolone, Fluazacort, Flucloronide, Flumethasone, Flunisolide, Flucinolone Acetonide, Fluocinonide, Fluocortin Butyl, Fluocortolone, Fluorometholone, Fluperolone Acetate, Fluprednidene Acetate, Fluprednisolone, Flurandrenolide, Fluticasone Propionate, Formocortal, Halcinonide, Halobetasol Propionate, Halometasone, Halopredone Acetate, Hydrocortamate, Hydrocortisone, Loteprednol Etabonate, Mazipredone, Medrysone, Meprednisone, Methylprednisolone, Mometasone Furoate, Paramethasone, Prednicarbate, Prednisolone, Prednisolone 25-Diethylaminoacetate, Predisolone Sodium Phosphate, Prednisone, Prednival, Prednylidene, Rimexolone, Tixocortol, Triamcinolone, Triameinolone, Acetonide, Triamcinolone Benetonide, Triamcinolone Hexacetonide.

42. A method of treating a MIF-mediated disease comprising the step of administering a pharmaceutically acceptable amount of the composition of Claim 37.

43. A method of treating an inflammatory disease comprising administering a therapeutically effective amount of an anti-MIF antibody according to Claim 7.

44. The method of Claim 43 wherein said disease is selected from the group consisting of arthritis, psoriasis, glomerulonephritis, septic shock, and atopic dermatitis.

45. A method of inhibiting angiogenesis comprising administering a therapeutically effective amount of an anti-MIF antibody according to Claim 7.

46. A method of treating cancer comprising administering a therapeutically effective amount of an anti-MIF antibody according to Claim 7.

47. The method of Claiml 46 wherein said antibody inhibits angiogenesis.

48. The method of Claim 43 wherein the treated disease is septic shock.

49. The method of Claim 43 wherein the treated disease is glomerulonephritis.

50. The method of Claim 43 wherein the treated disease is rheumatoid arthritis.

51. The method of Claim 43 wherein the treated disease is atopic dermatitis.

52. The method of Claim 42 wherein the treated disease is retinopathy.

53. The method of Claim 52 wherein retinopathy is associated with diabetes or lupus.

FIG. 1A

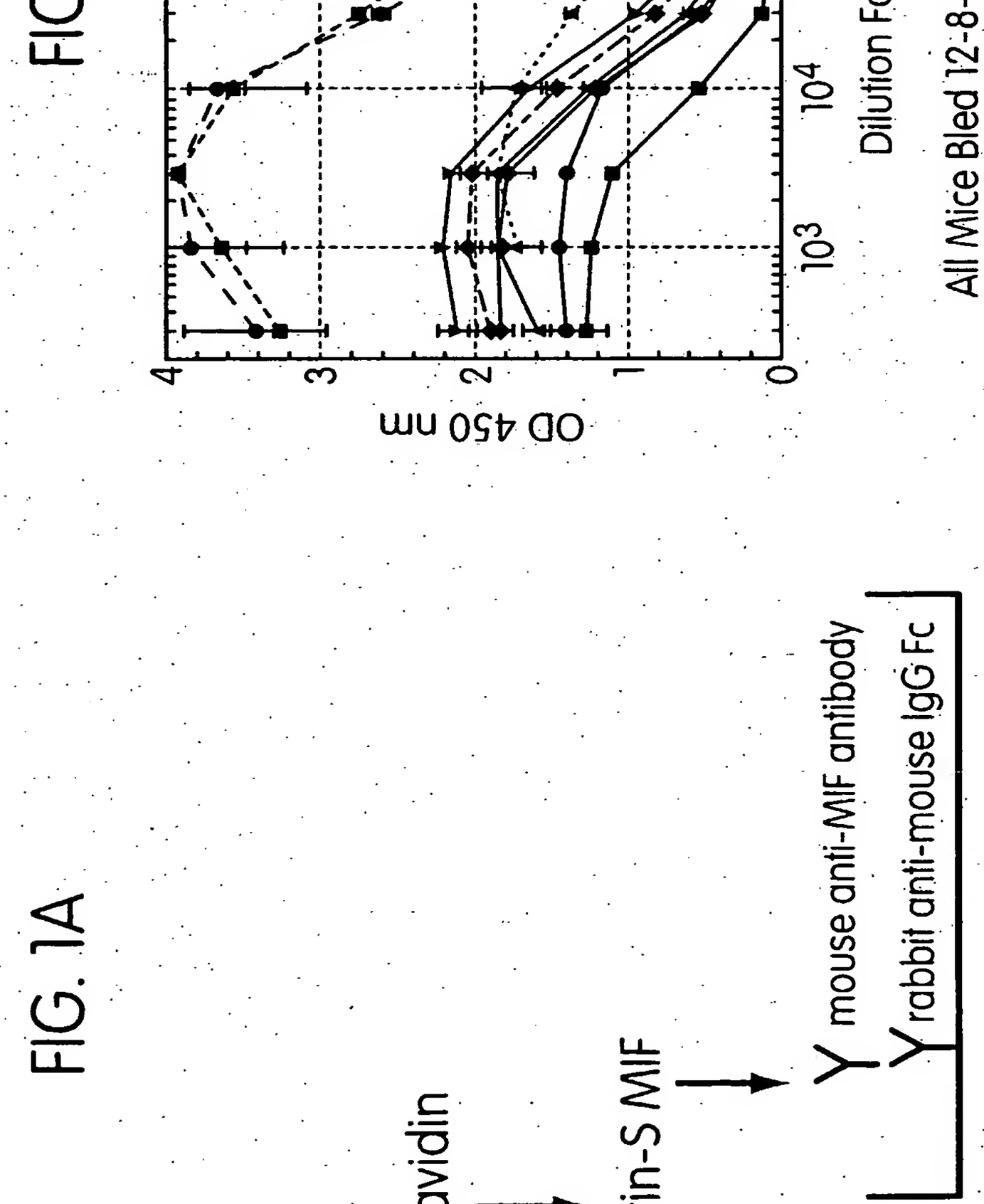


FIG. 1B

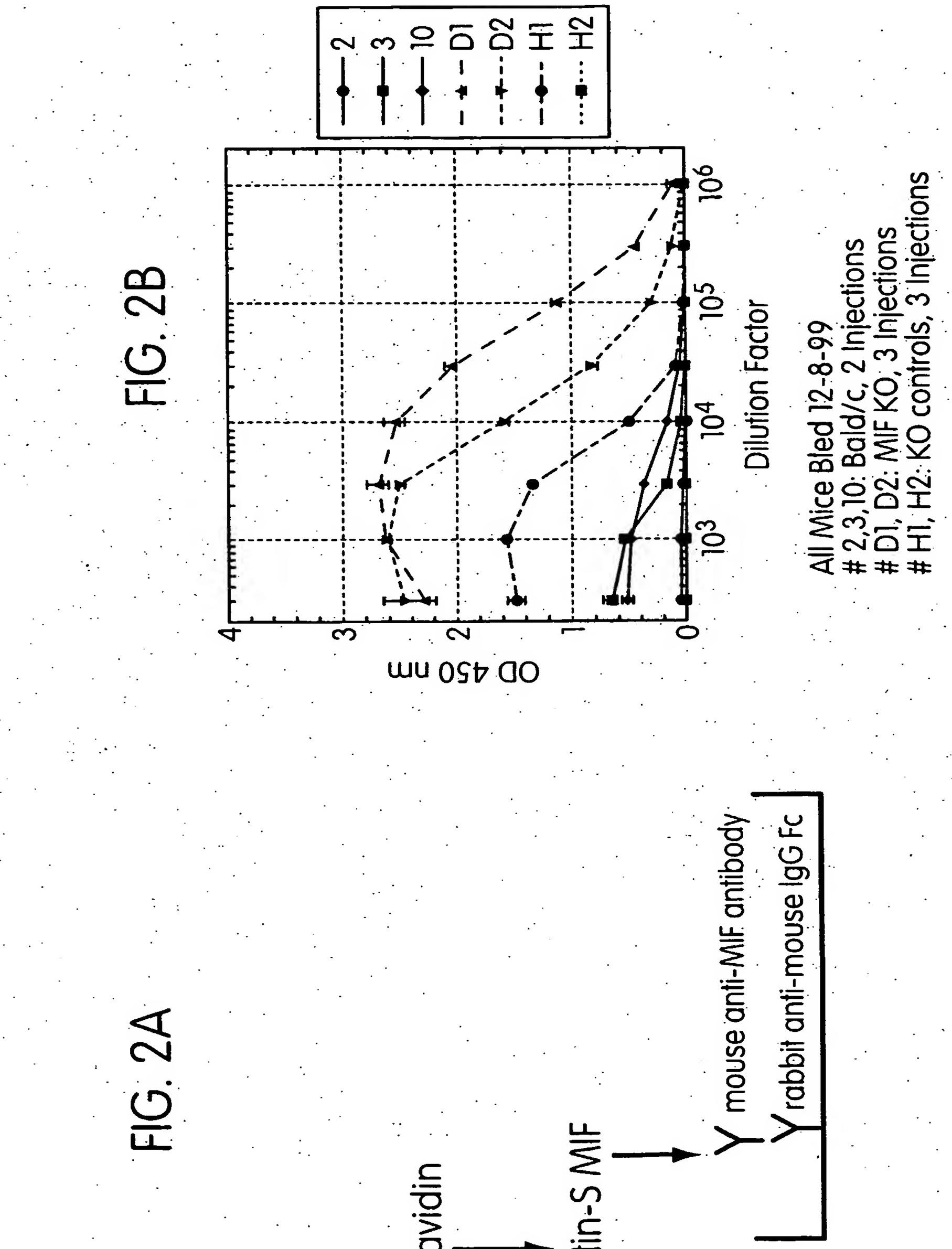


FIG. 2B

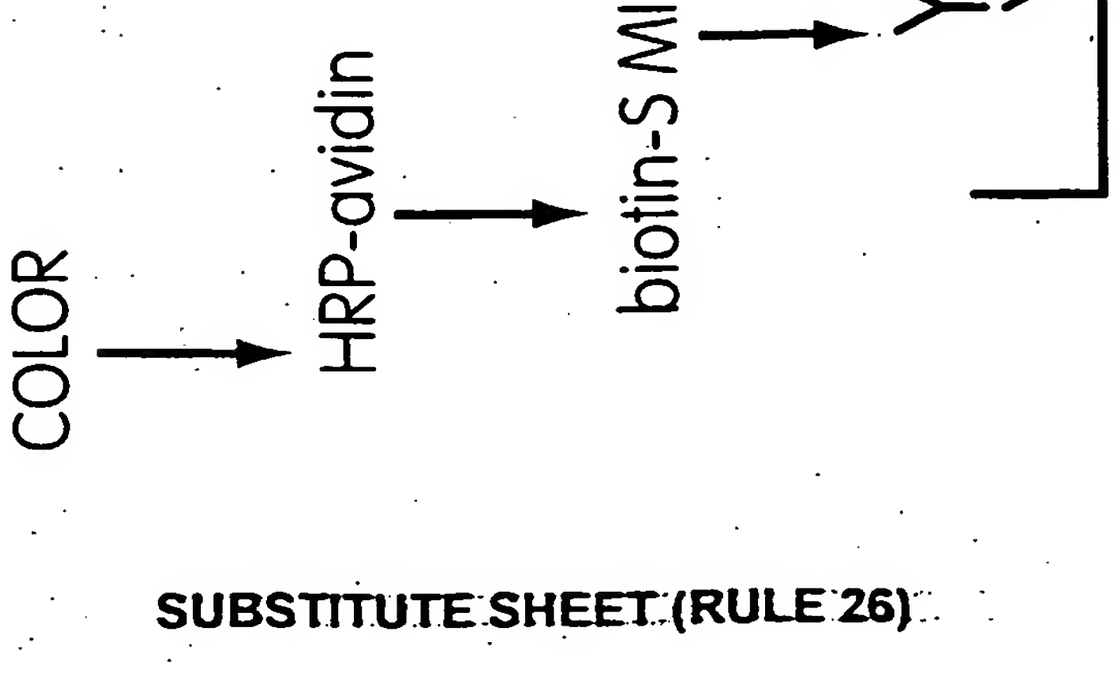


FIG. 2A

FIG. 3A

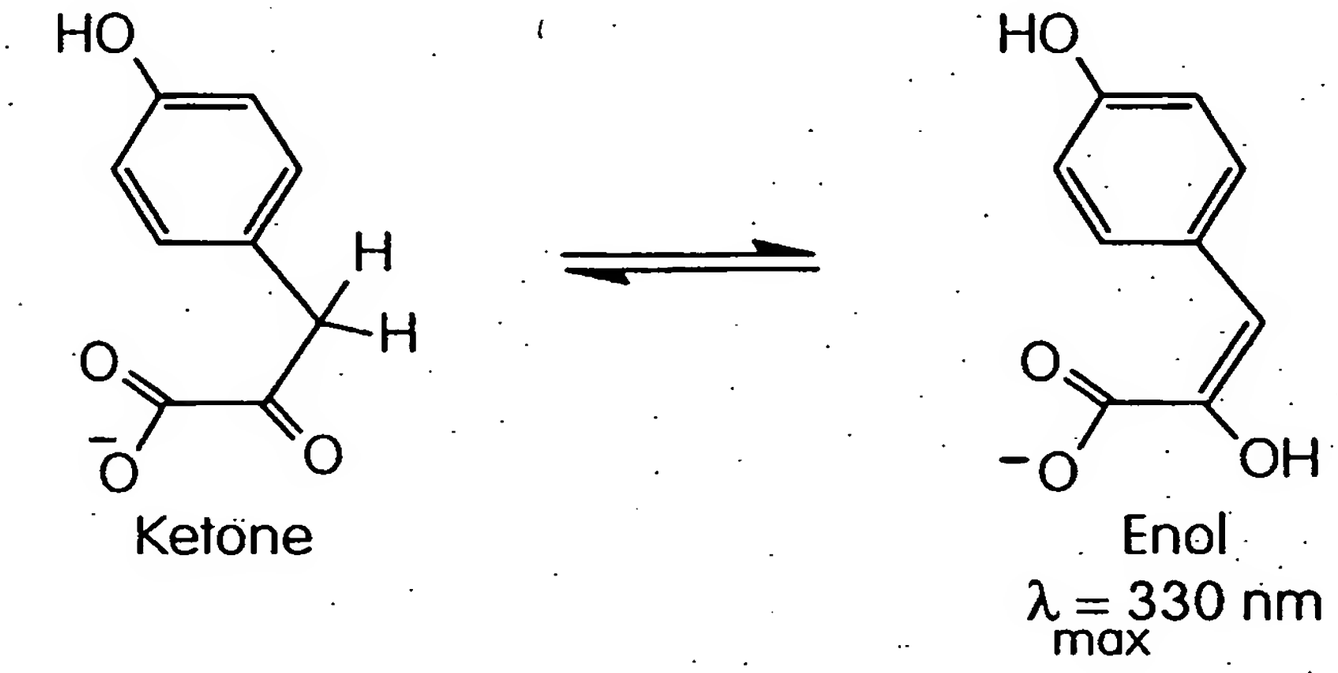


FIG. 3B

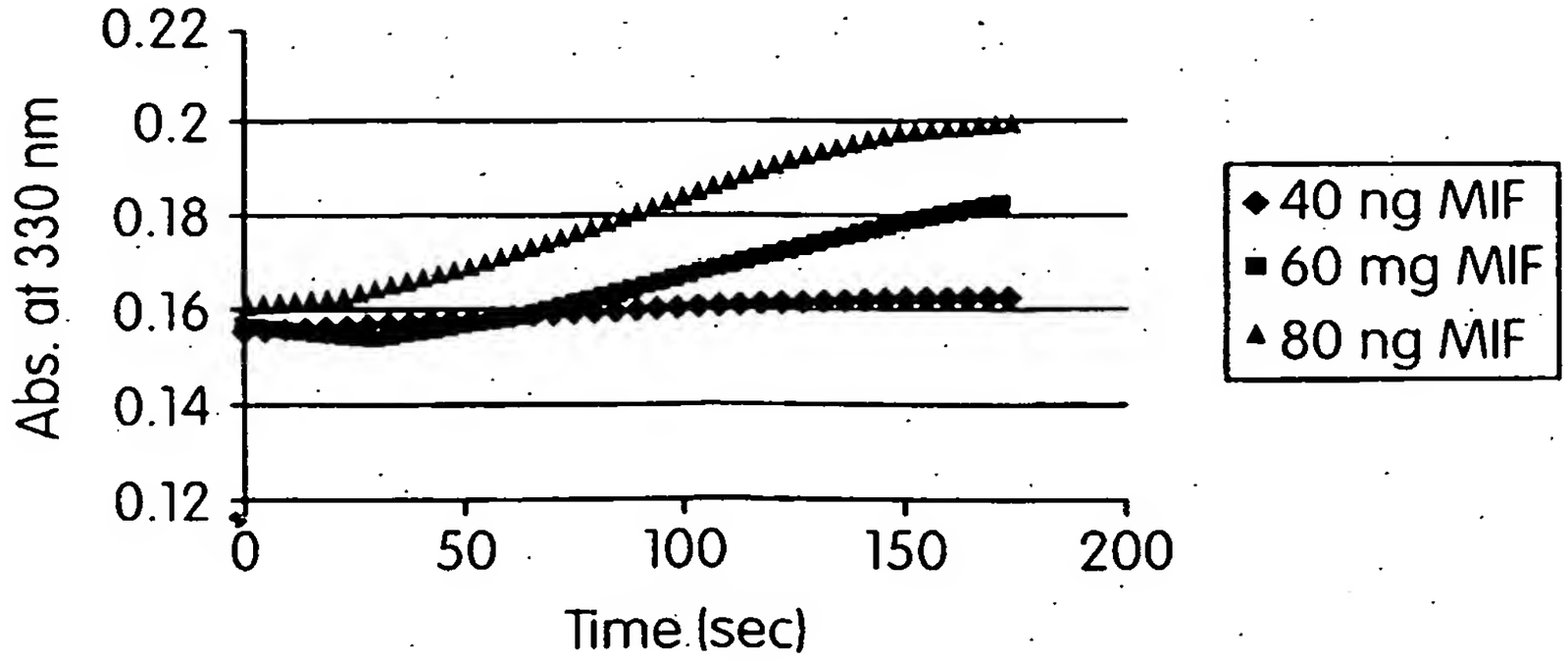


FIG. 4

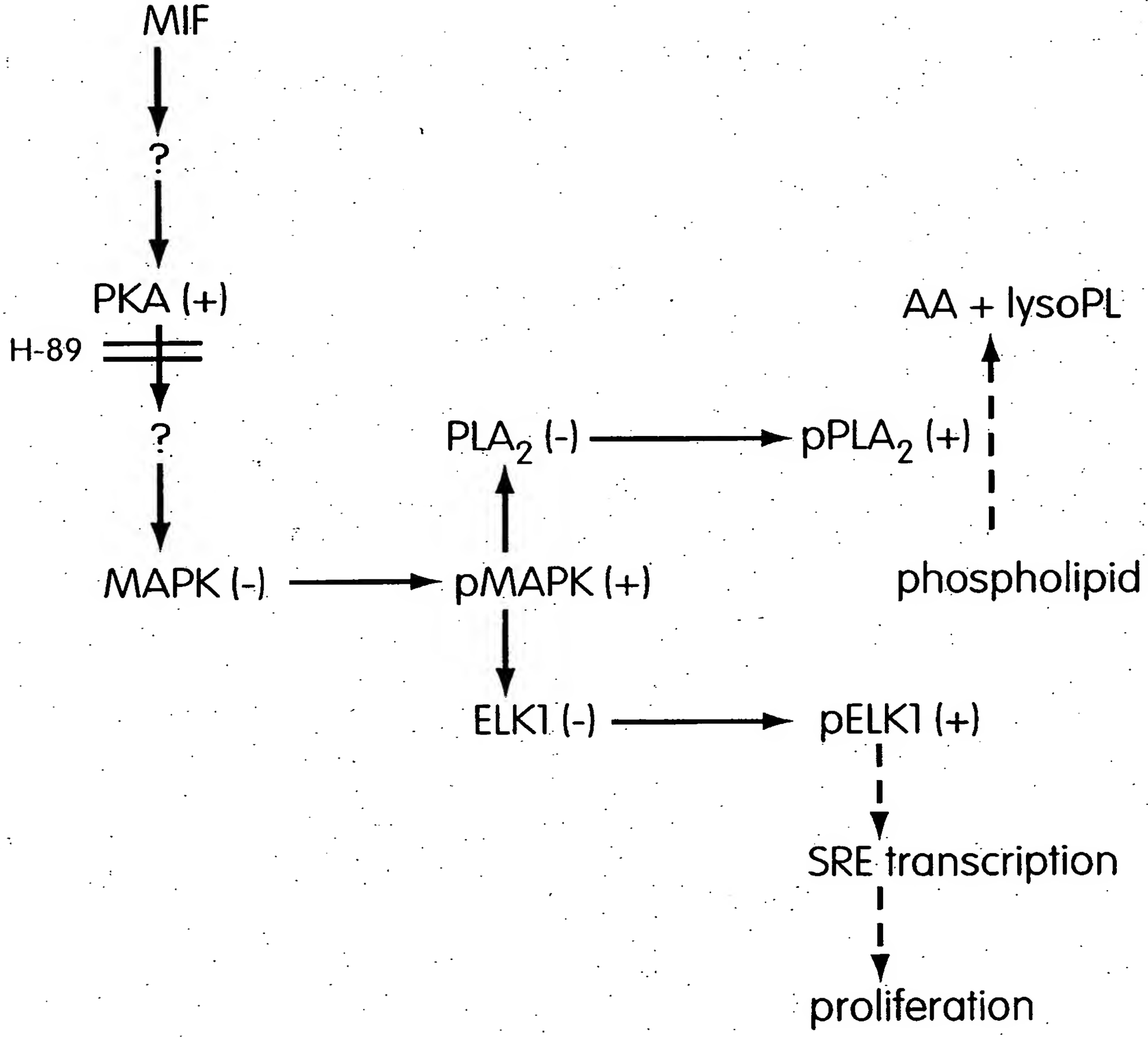


FIG. 5

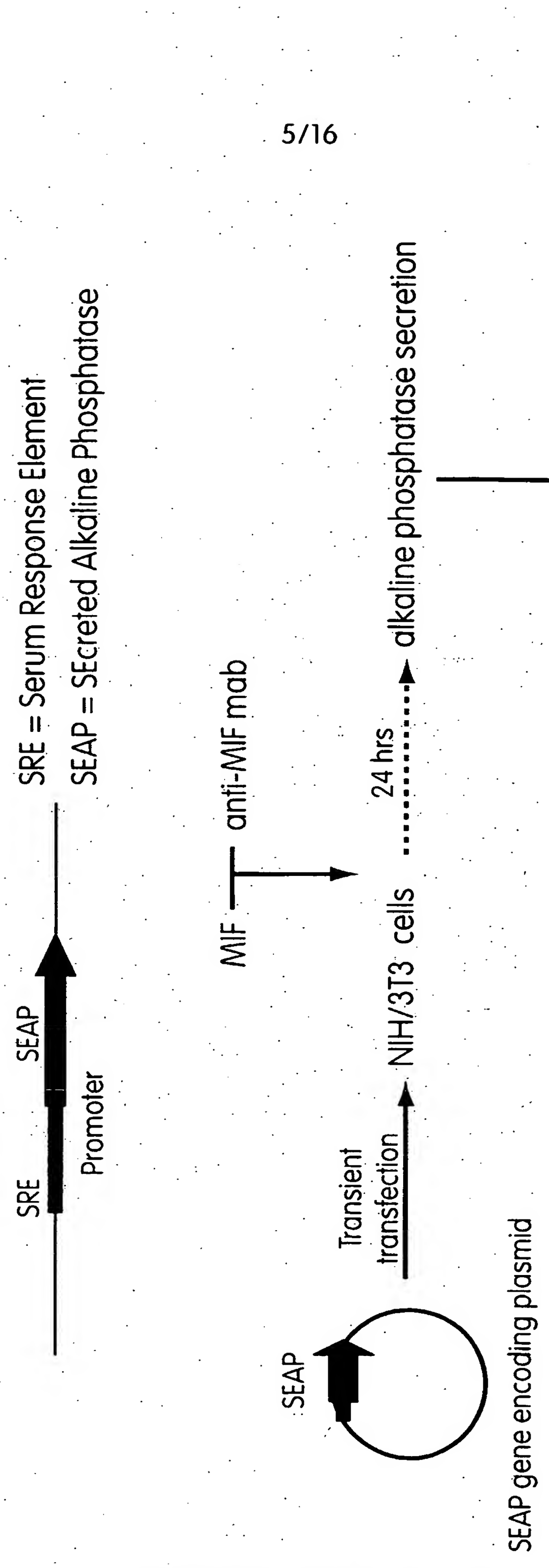
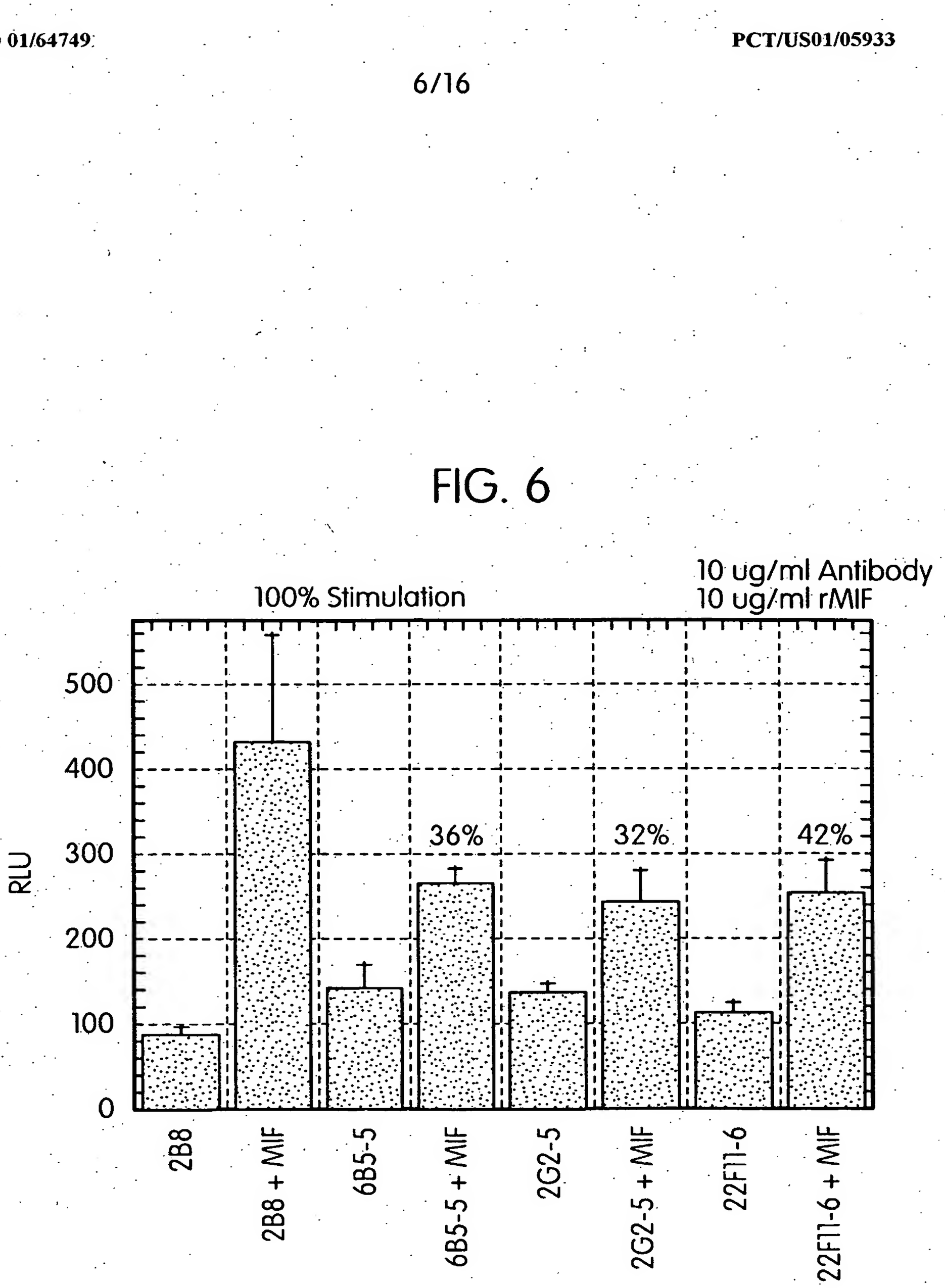


FIG. 6



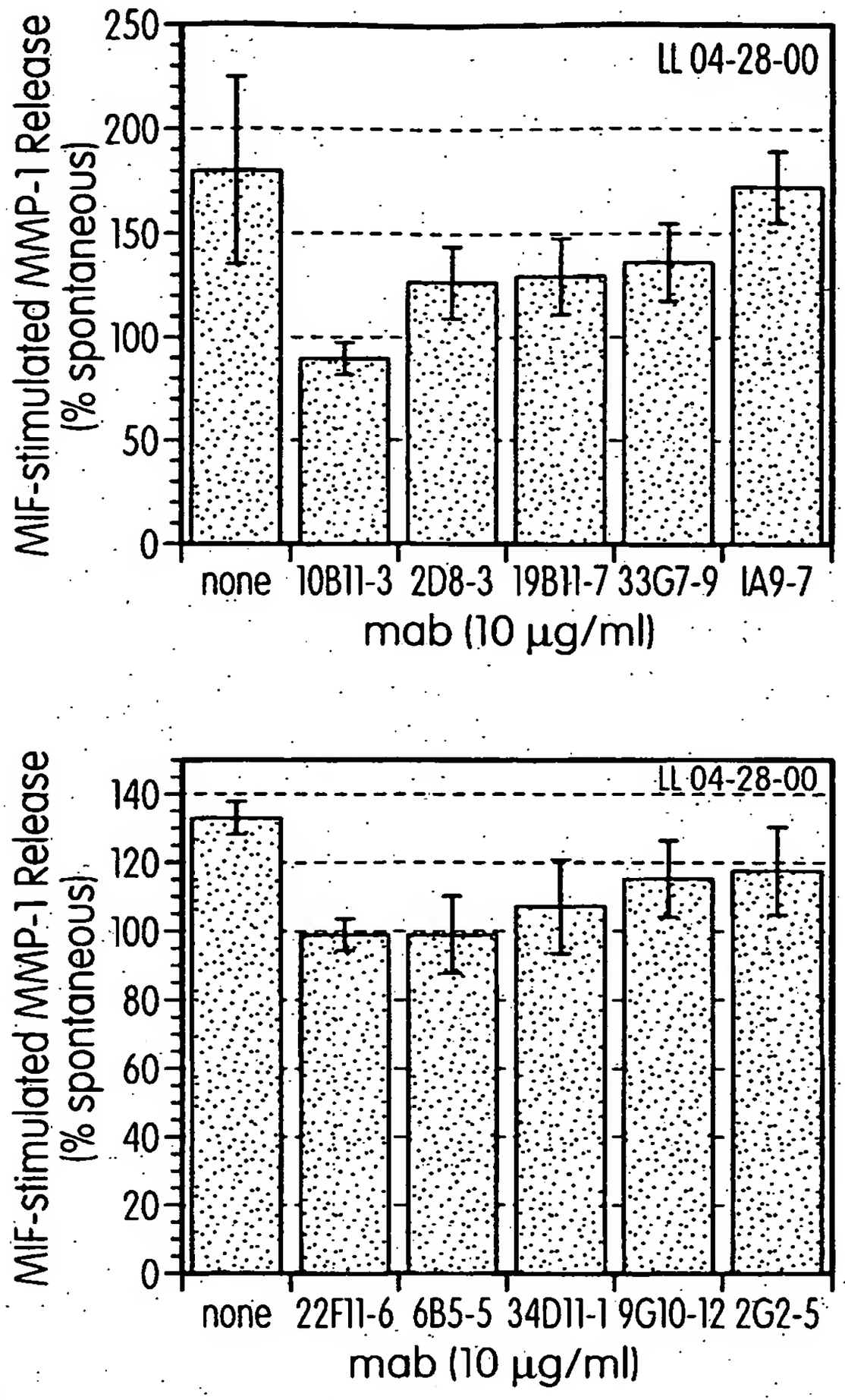


FIG. 7A

FIG. 7B

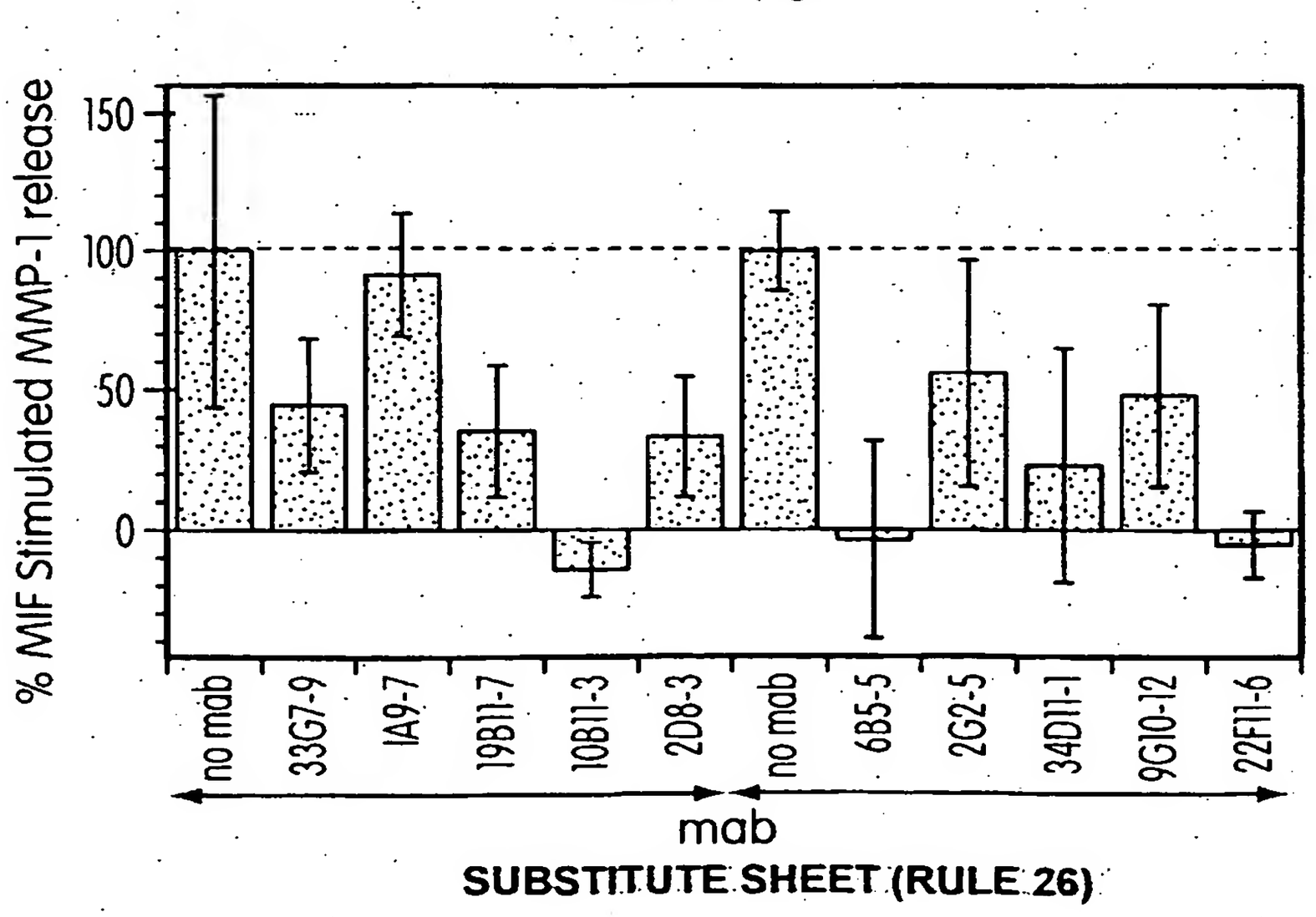


FIG. 7C

FIG. 8A

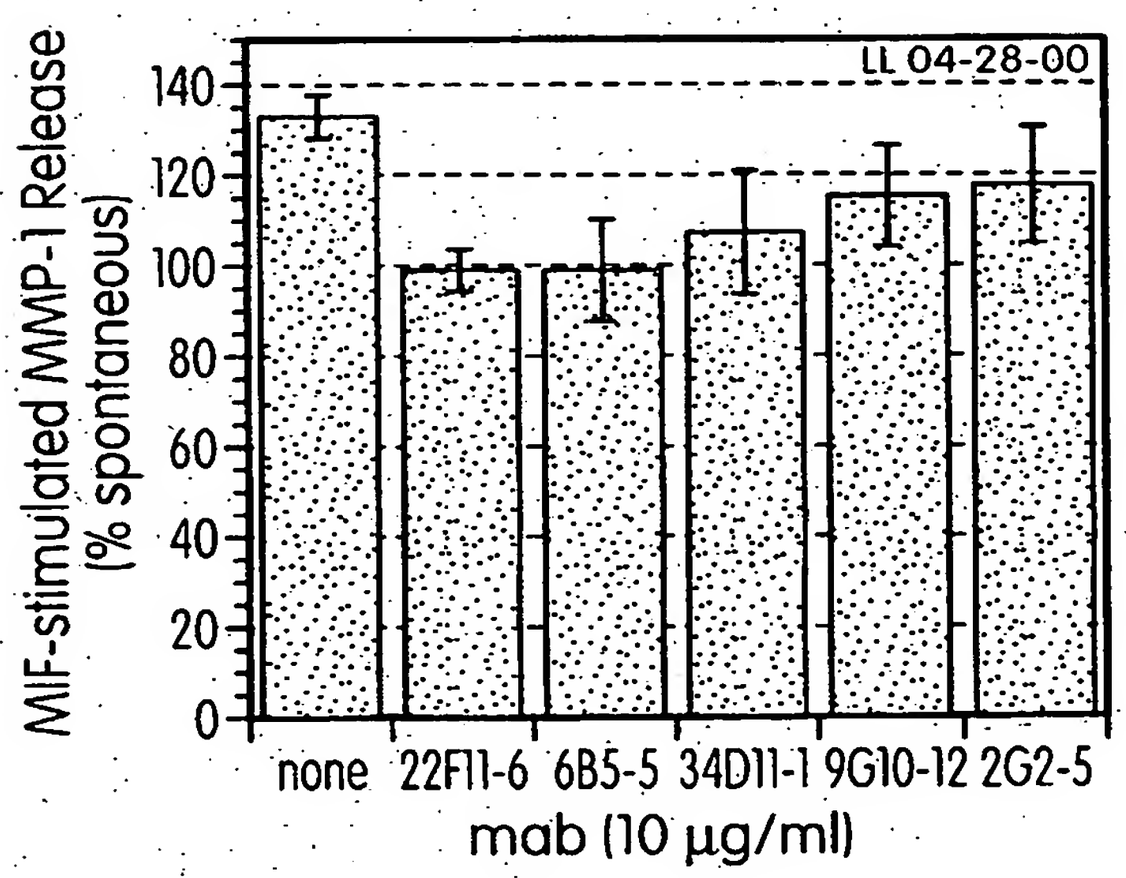


FIG. 8B

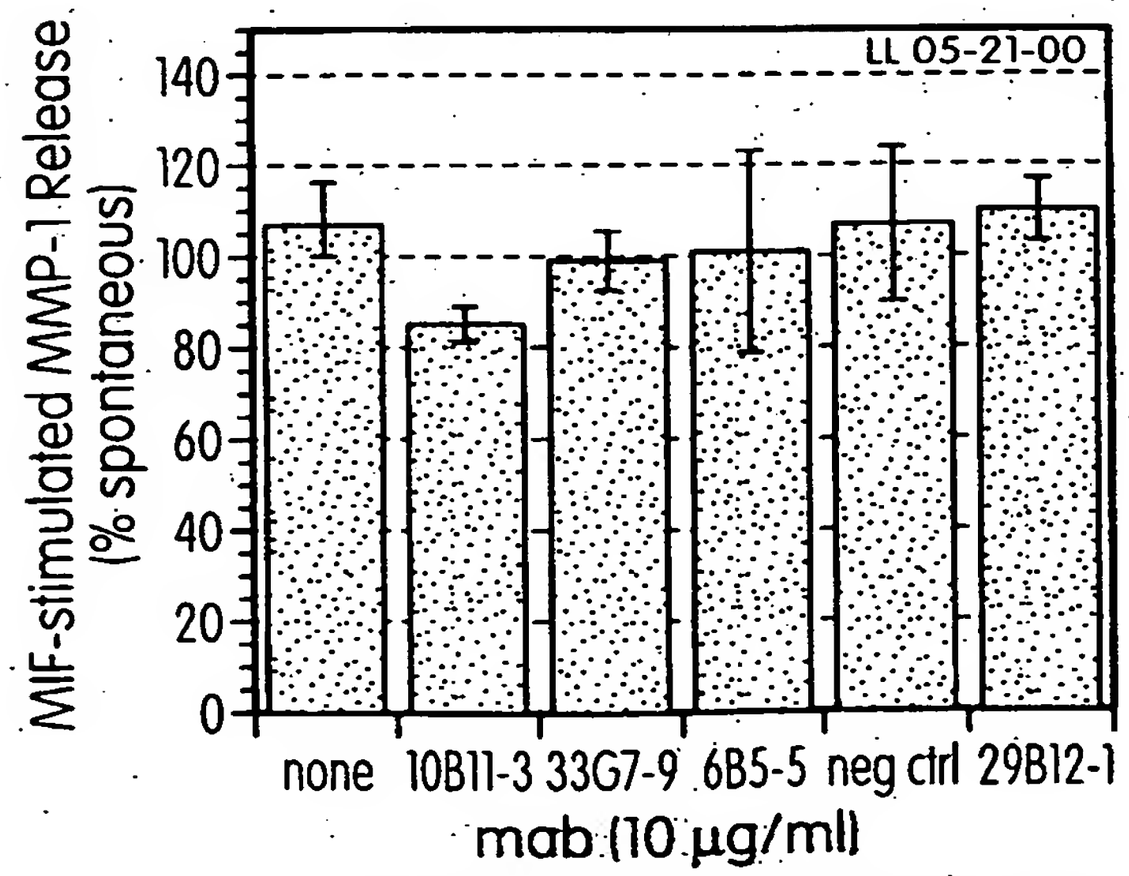


FIG. 8C

FIG. 9

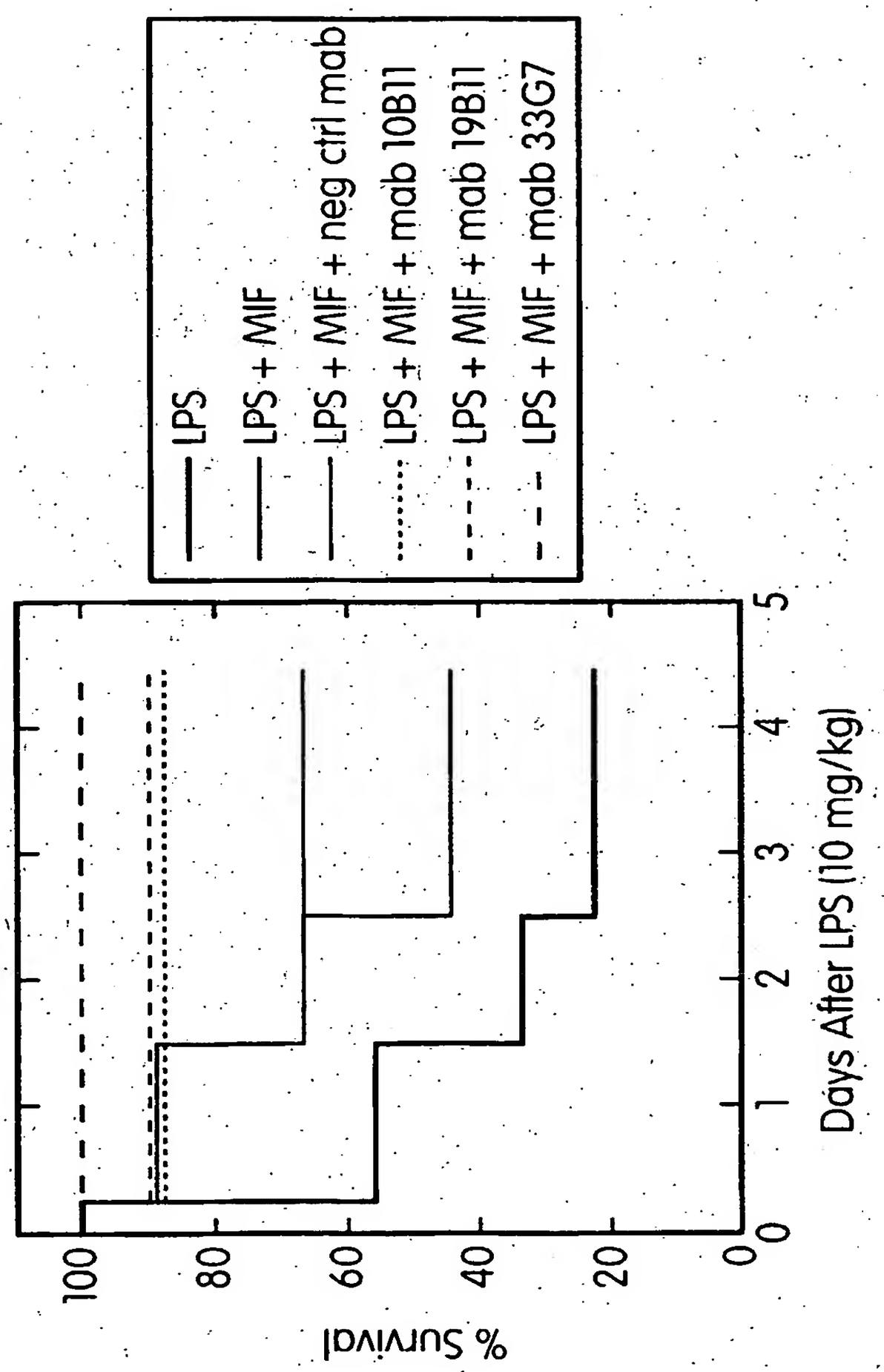


FIG.10

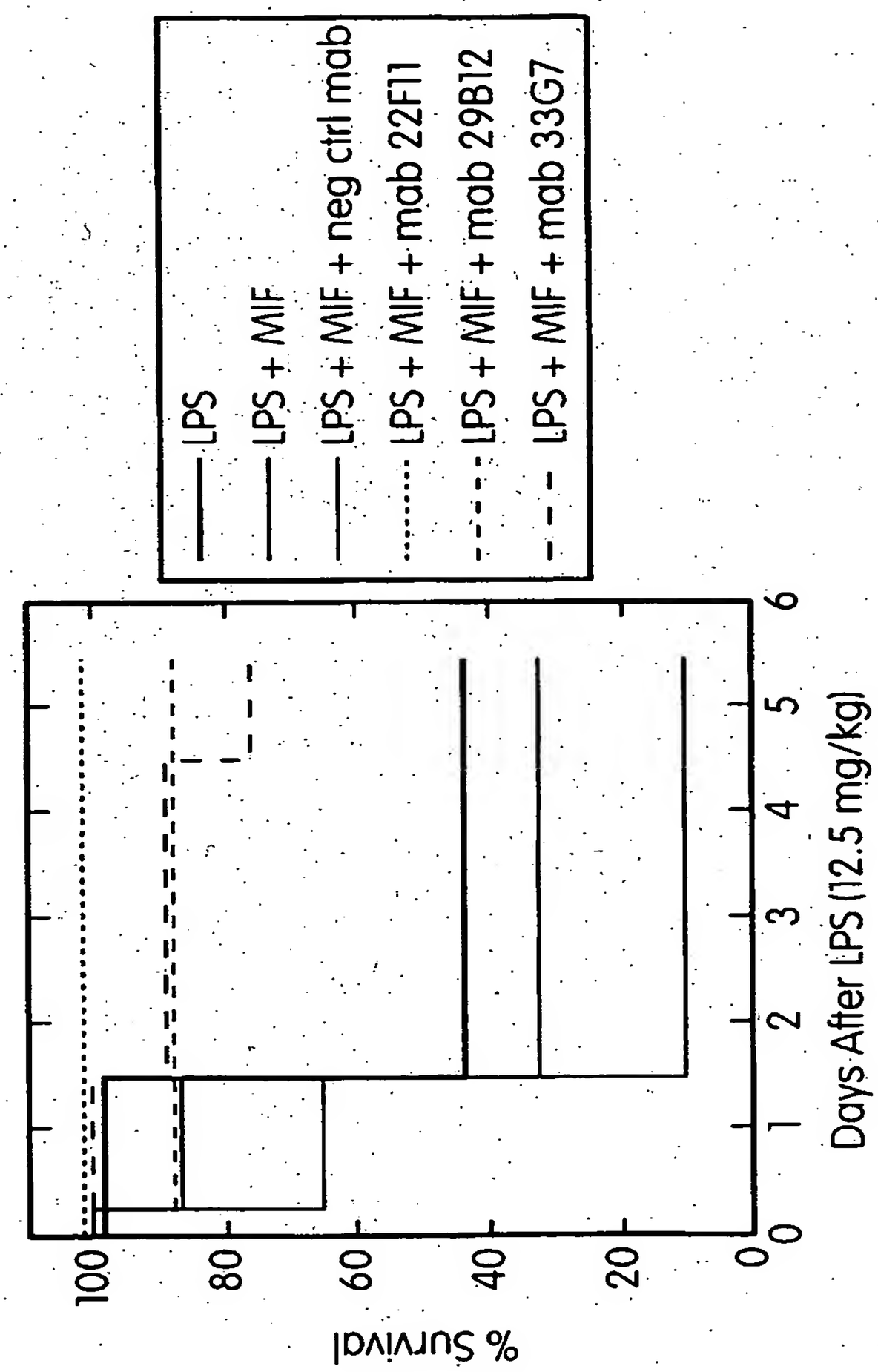


FIG. 11

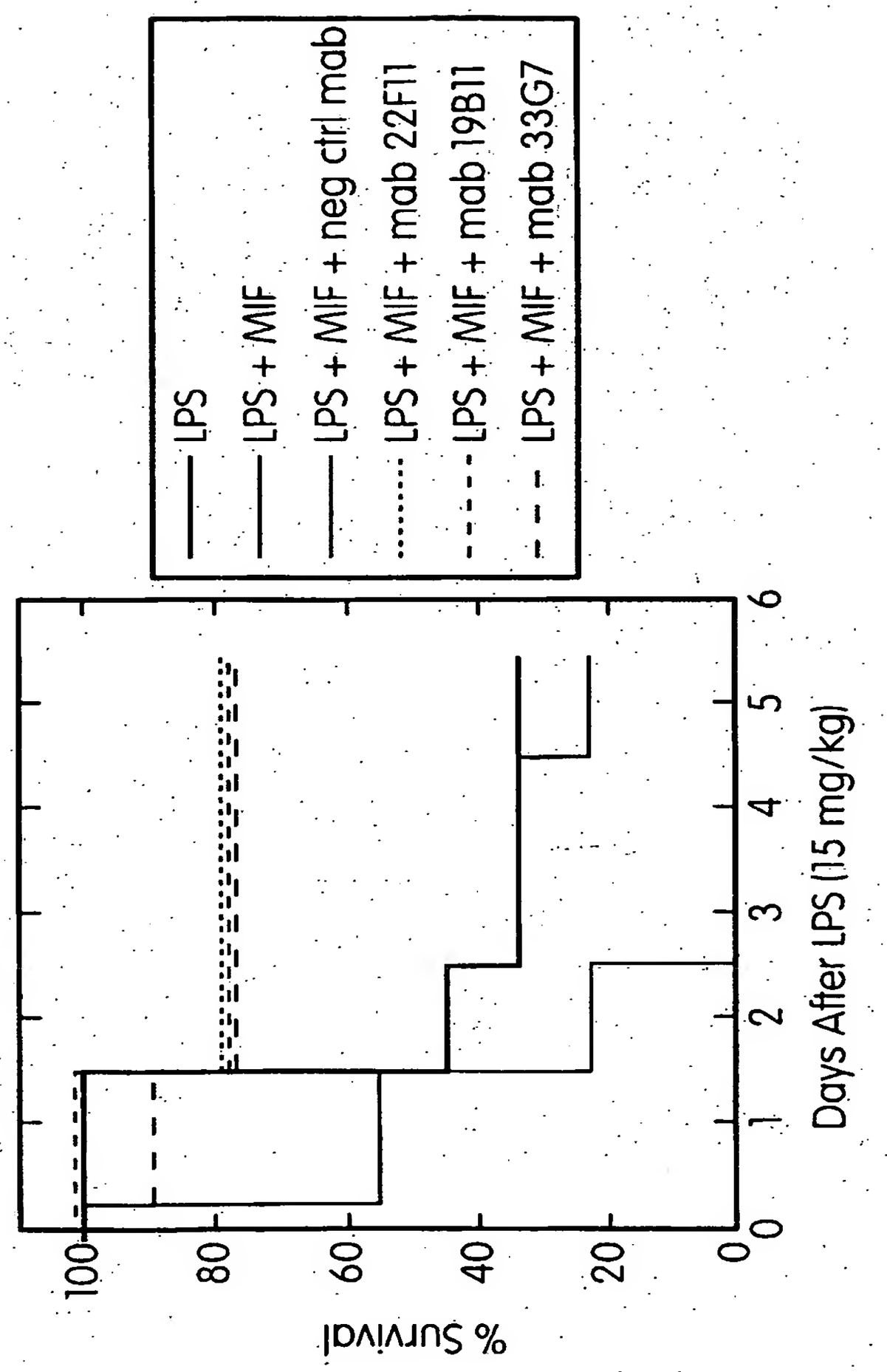


FIG. 12A

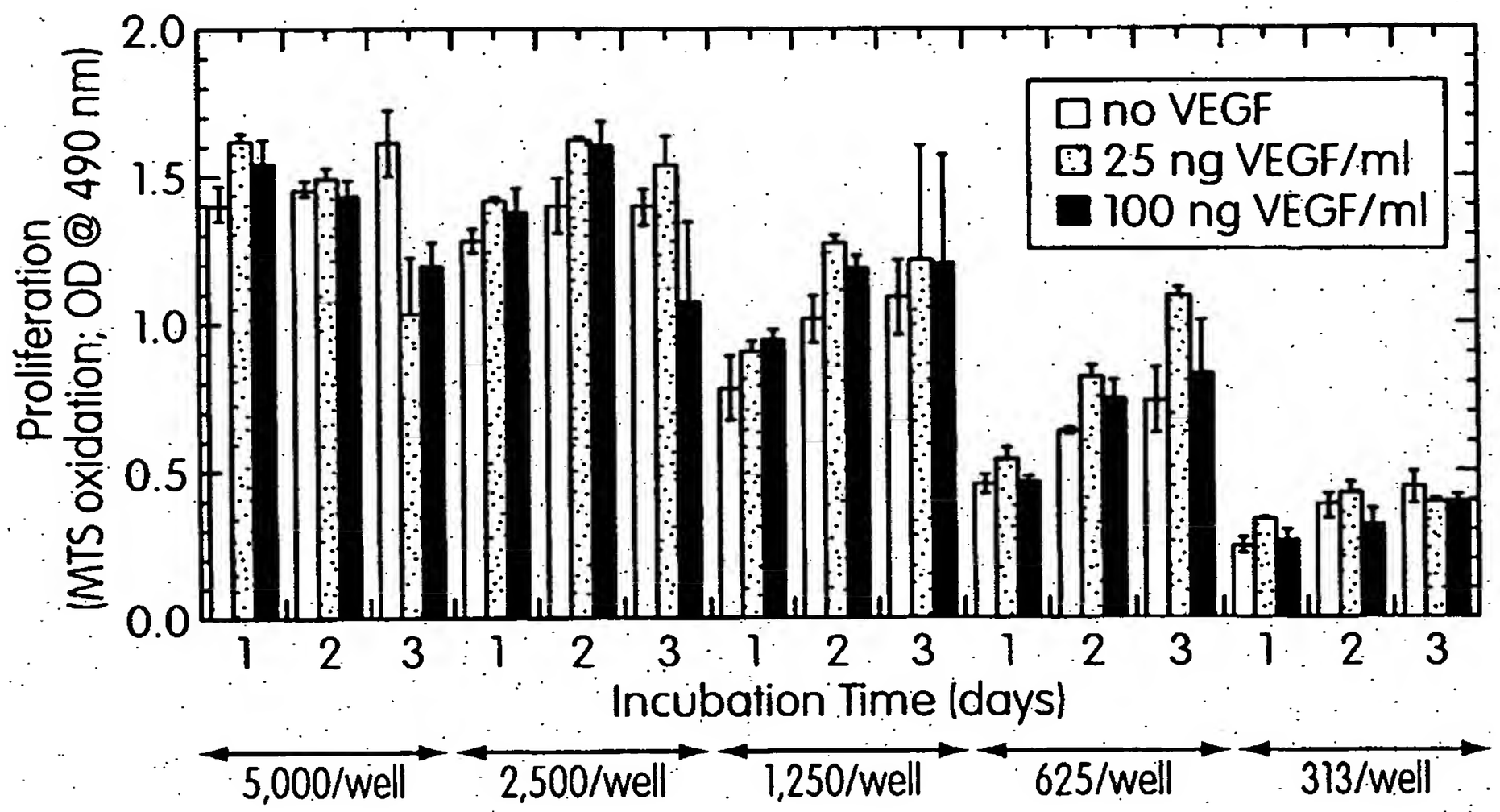


FIG. 12B

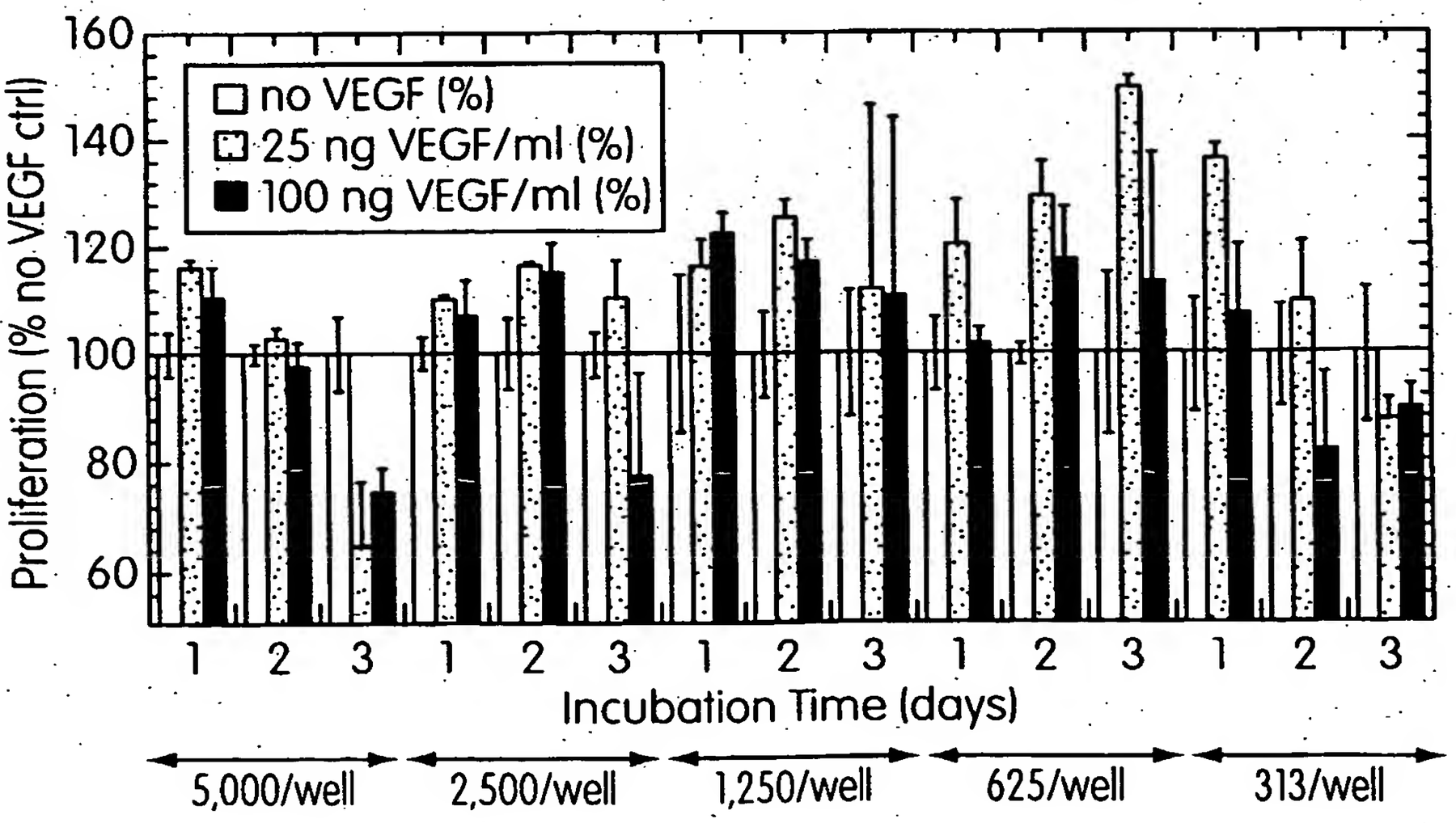


FIG. 13

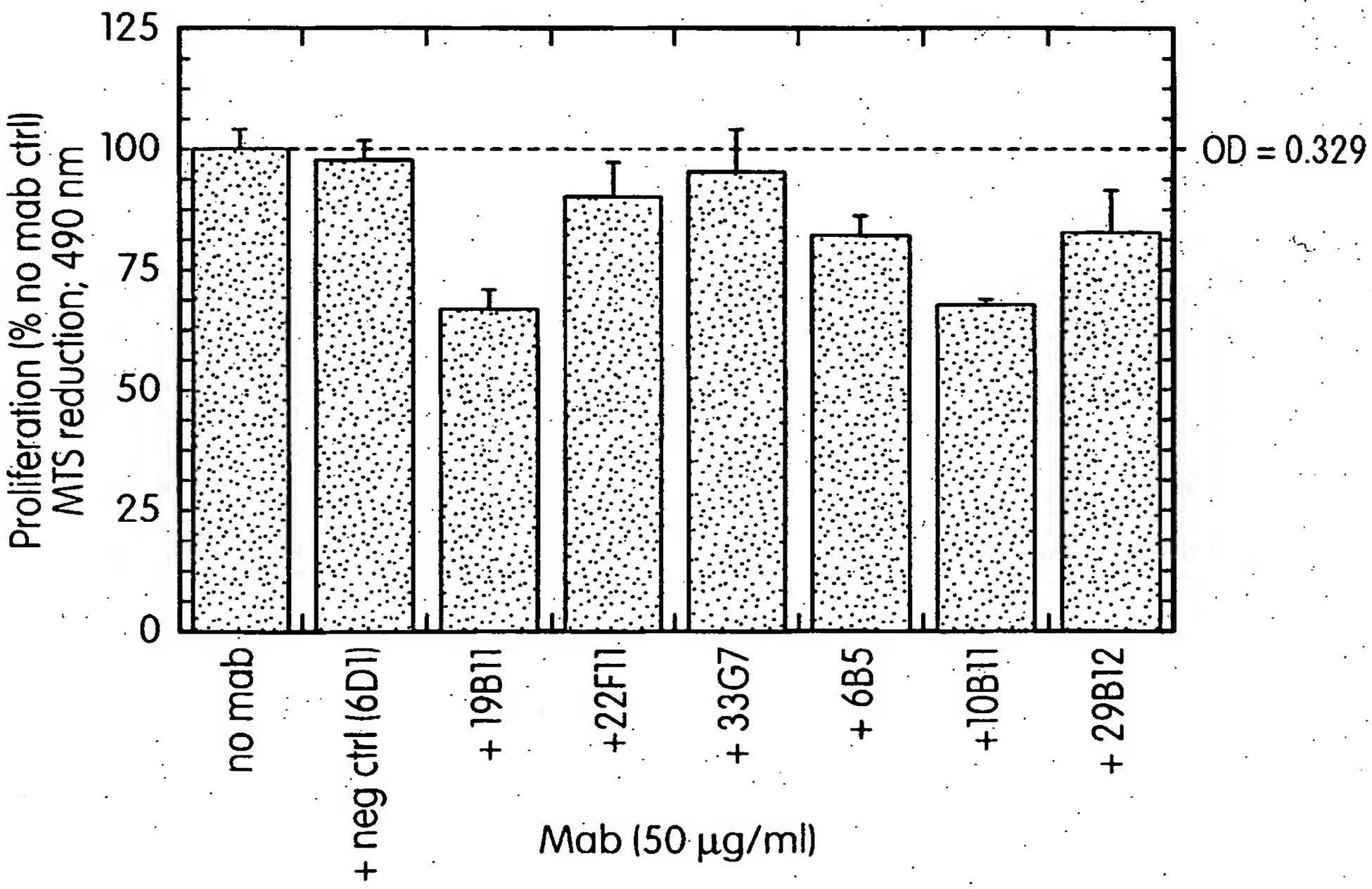


FIG. 14

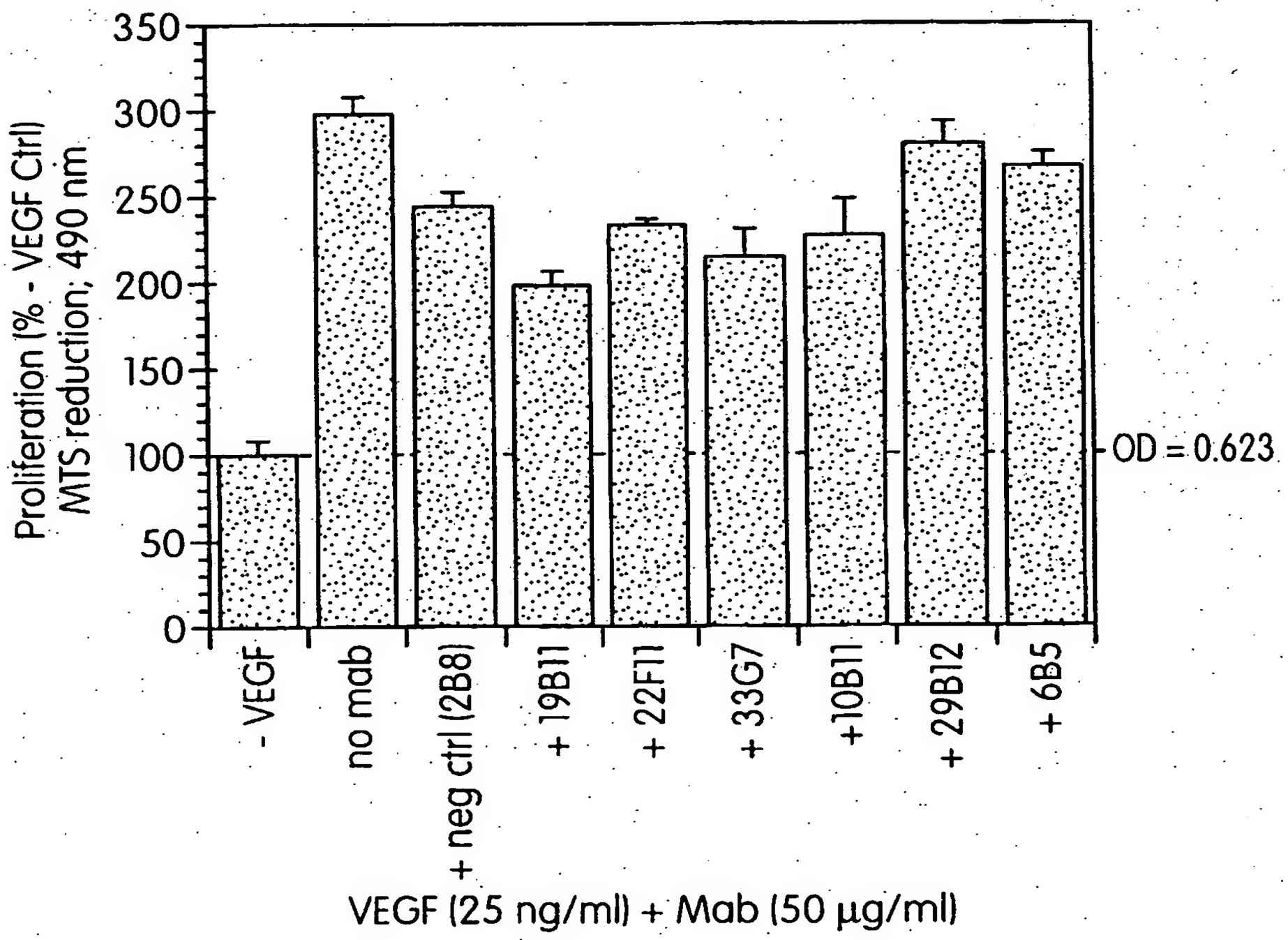
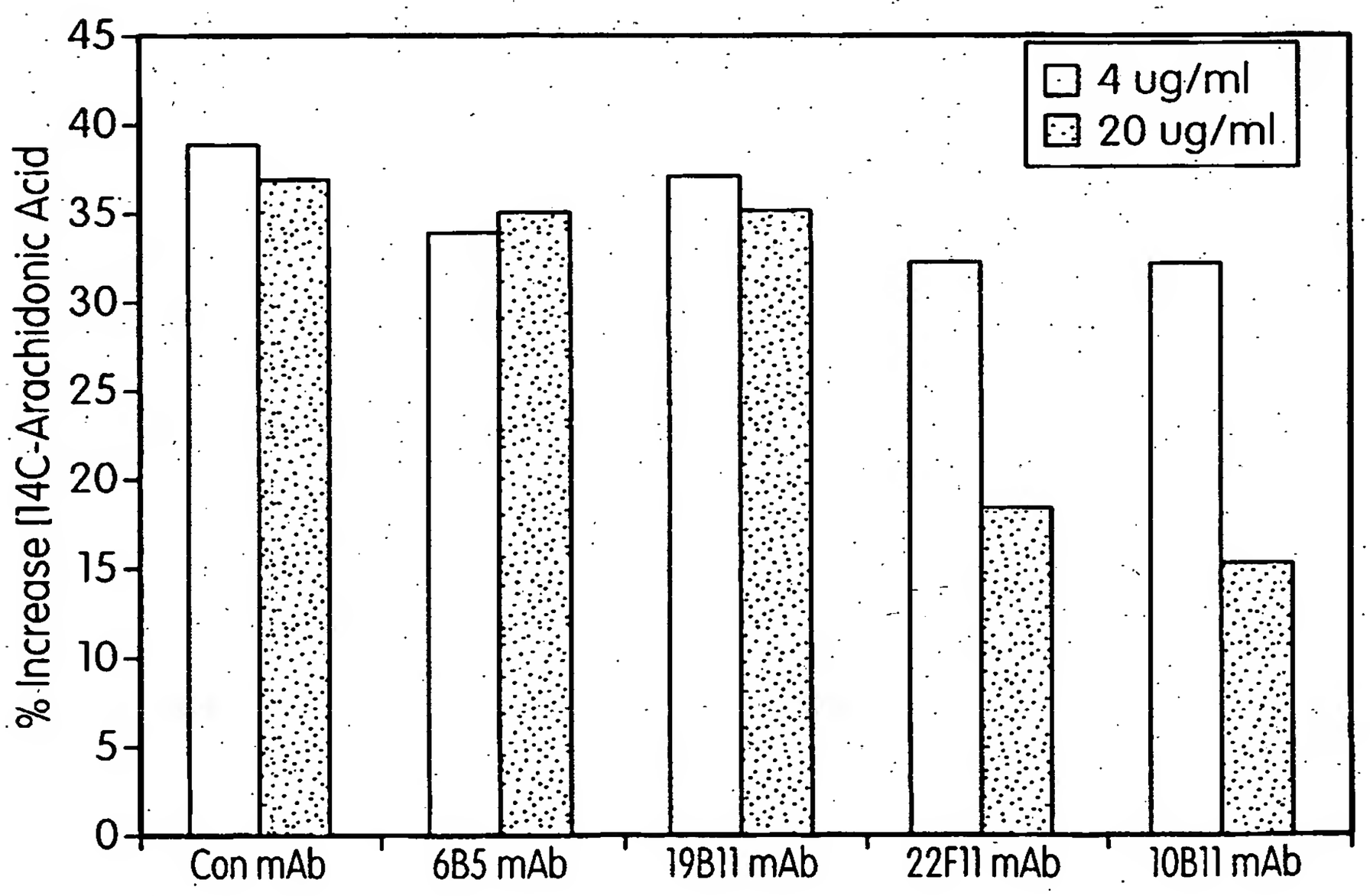


FIG. 15



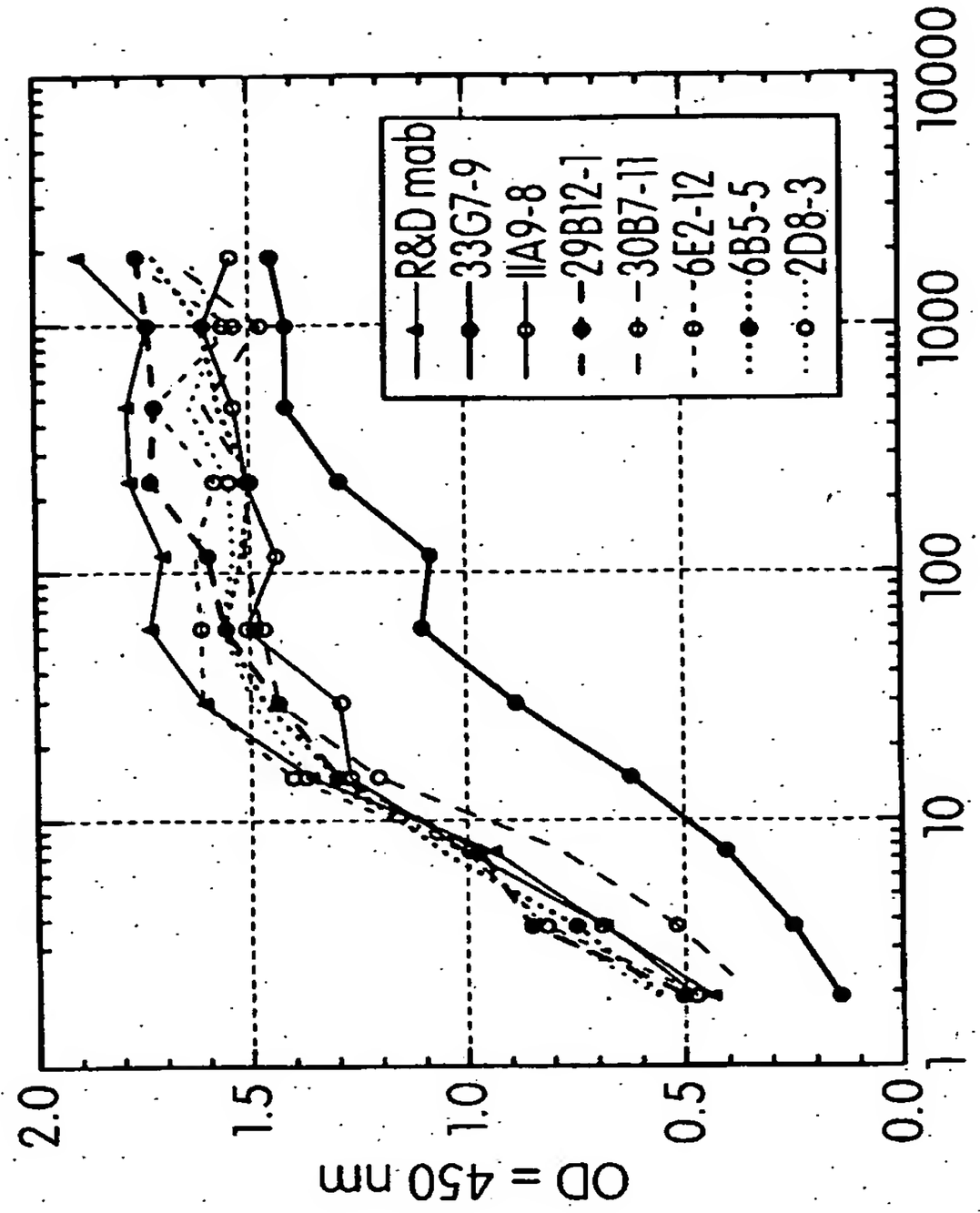


FIG. 16B

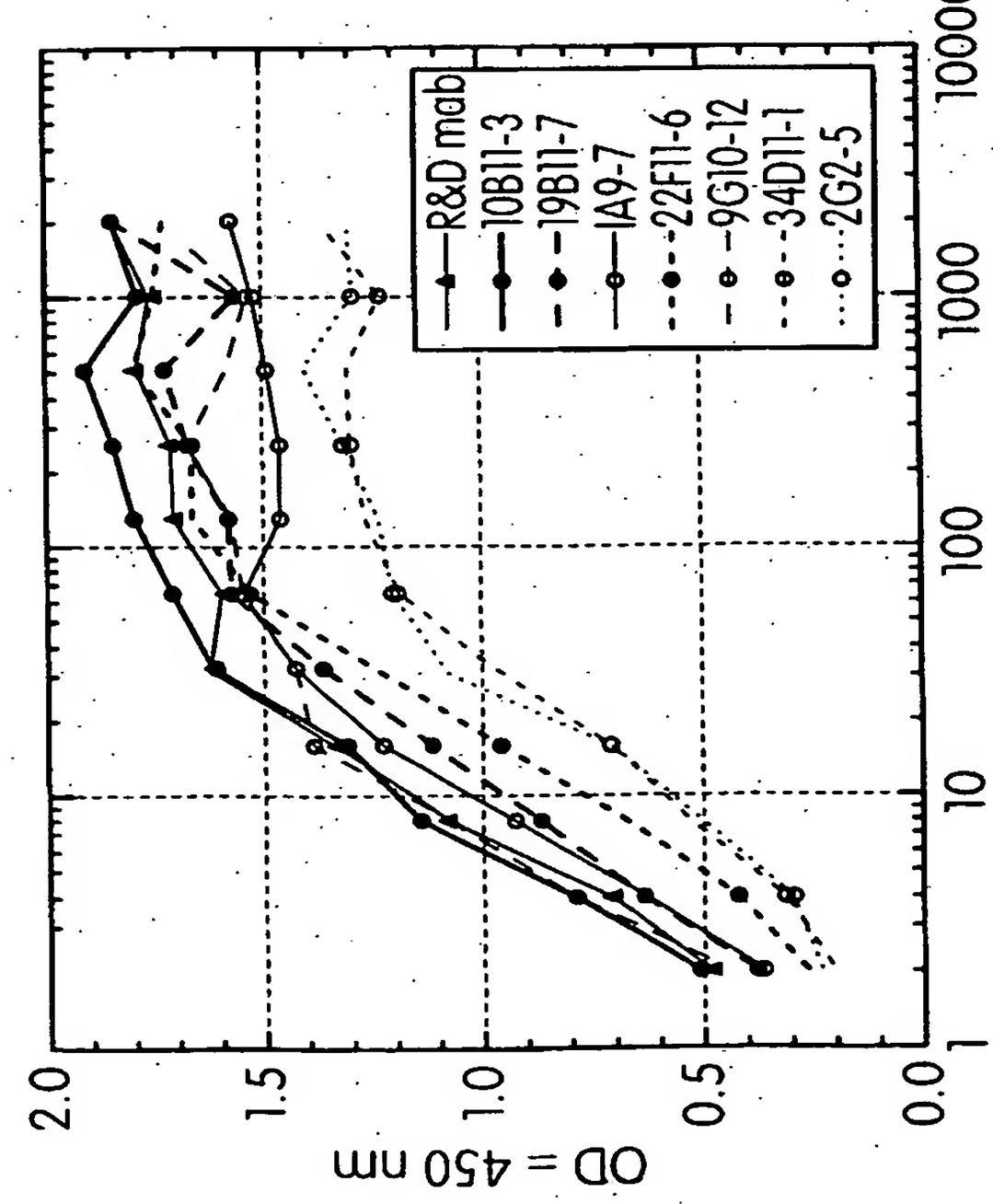


FIG. 16A

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
7 September 2001 (07.09.2001)

PCT

(10) International Publication Number
WO 01/64749 A3

(51) International Patent Classification[7]: A01K 67/027, C07K 16/00, A61K 39/395, C12N 15/12, 15/06, 15/13, 5/10, 15/79, A61K 31/56, 31/00, 38/00, A61P 29/00, 13/12, 17/06, 19/02, 27/02, 35/00, 31/04 // C07K 16/24, C12N 5/20, (A61K 39/395, 38:00) (A61K 39/395, 31:00) (A61K 39/395, 31:56)

(21) International Application Number: PCT/US01/05933

(22) International Filing Date: 26 February 2001 (26.02.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/185,390 28 February 2000 (28.02.2000) US
60/233,625 18 September 2000 (18.09.2000) US

(71) Applicant: IDEC PHARMACEUTICALS CORPORATION [US/US]; 11011 Torreyana Road, San Diego, CA 92121 (US).

(72) Inventors: KLOETZER, William, S.; 4317 Sea Bright Drive, Carlsbad, CA 92008 (US). HANNA, Nabil; 14770 Avenida Insurgentes, Rancho Santa Fe, CA 92067 (US).

(74) Agents: TESKIN, Robin, L. et al.; Pillsbury Winthrop LLP, 1600 Tysons Boulevard, McLean, VA 22102 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
2 May 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/64749 A3

(54) Title: METHOD FOR PREPARING ANTI-MIF ANTIBODIES

(57) Abstract: The specification provides methods of preparing high-affinity antibodies to a macrophage migration inhibitory factor (MIF) in animals in which the *MIF* gene has been homozygously knocked-out ($MIF^{-/-}$). Also provided are methods of preparing hybridomas which produce the anti-MIF antibodies, methods of administering the antibodies to treat inflammatory or cancerous conditions and/or diseases modulated by MIF, as well as compositions comprising said high-affinity anti-MIF antibodies.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/05933

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A01K67/027 C07K16/00 A61K39/395 C12N15/12 C12N15/06
C12N15/13 C12N5/10 C12N15/79 A61K31/56 A61K31/00
A61K38/00 A61P29/00 A61P13/12 A61P17/06 A61P19/02
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A01K C07K A61K C12N A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EPO-Internal, MEDLINE, WPI Data, PAJ, CHEM ABS Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BOZZA MARCELO ET AL: "Targeted disruption of migration inhibitory factor gene reveals its critical role in sepsis." JOURNAL OF EXPERIMENTAL MEDICINE, vol. 189, no. 2, 18 January 1999 (1999-01-18), pages 341-346, XP002174629 ISSN: 0022-1007 cited in the application	26-35
Y	the whole document	1-25, 27-53

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 August 2001	03/09/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Renggli, J

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/05933

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61P27/02 A61P35/00 A61P31/04 //C07K16/24,C12N5/20,
(A61K39/395,38:00),(A61K39/395,31:00),(A61K39/395,31:56)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DECLERCK PAUL J ET AL: "Generation of monoclonal antibodies against autologous proteins in gene-inactivated mice." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 15, 1995, pages 8397-8400, XP002174630 ISSN: 0021-9258 cited in the application the whole document --- -/--	1-25, 27-53

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 August 2001	

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Renggli, J

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter · ·nal Application No
PCT/US 01/05933

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication where appropriate, of the relevant passages	Relevant to claim No.
X	STEINHOFF M ET AL: "Evidence for a role of macrophage migration inhibitory factor in psoriatic skin disease." BRITISH JOURNAL OF DERMATOLOGY, vol. 141, no. 6, December 1999 (1999-12), pages 1061-1066, XP002174631 ISSN: 0007-0963 cited in the application the whole document	7-17, 20-23, 25,36-44
X	DONNELLY S C ET AL: "REGULATORY ROLE FOR MACROPHAGE MIGRATION INHIBITORY FACTOR IN ACUTERESPIRATORY DISTRESS SYNDROME" NATURE MEDICINE, NATURE AMERICA, NEW YORK, US, vol. 3, no. 3, March 1997 (1997-03), pages 320-323, XP000885655 ISSN: 1078-8956 the whole document	7-17, 20-23, 25,36-44
X	CALANDRA THIERRY ET AL: "Protection from septic shock by neutralization of macrophage migration inhibitory factor." NATURE MEDICINE, vol. 6, no. 2, February 2000 (2000-02), pages 164-170, XP002174632 ISSN: 1078-8956 cited in the application the whole document	7-17, 20-23, 25, 36-44,48
X	CALANDRA T ET AL: "MACROPHAGE MIGRATION INHIBITORY FACTOR: A COUNTER-REGULATOR OF GLUCOCORTICOID ACTION AND CRITICAL MEDIATOR OF SEPTIC SHOCK" JOURNAL OF INFLAMMATION, WILEY-LISS INC., NEW YORK, NY, US, vol. 47, no. 1/2, 1996, pages 39-51, XP000615263 ISSN: 1078-7852 cited in the application the whole document	7-17, 20-23, 25, 36-44,48
X	LAN H ET AL: "THE PATHOGENIC ROLE OF MACROPHAGE MIGRATION INHIBITORY FACTOR IN IMMUNOLOGICALLY INDUCED KIDNEY DISEASE IN THE RAT" JOURNAL OF EXPERIMENTAL MEDICINE, TOKYO, JP, vol. 185, no. 8, April 1997 (1997-04), pages 1455-1465, XP000885678 ISSN: 0022-1007 cited in the application the whole document	7-17, 20-23, 25, 36-44,49

-/--

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter. ...nal Application No
PCT/US 01/05933

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LEECH MICHELLE ET AL: "Macrophage migration inhibitory factor in rheumatoid arthritis: Evidence of proinflammatory function and regulation by glucocorticoids." ARTHRITIS & RHEUMATISM, vol. 42, no. 8, August 1999 (1999-08), pages 1601-1608, XP002174633 ISSN: 0004-3591 cited in the application the whole document ---	7-17, 20-23, 25, 36-44,50
X	EP 0 162 812 A (CIBA GEIGY AG) 27 November 1985 (1985-11-27) cited in the application the whole document ---	7-17, 20-23, 25, 36-44, 46,50,51
X	MITCHELL ROBERT A ET AL: "Sustained mitogen-activated protein kinase (MAPK) and cytoplasmic phospholipase A2 activation by macrophage migration inhibitory factor (MIF): Regulatory role in cell proliferation and glucocorticoid action." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 274, no. 25, 18 June 1999 (1999-06-18), pages 18100-18106, XP002174634 ISSN: 0021-9258 cited in the application the whole document ---	7-17, 20-23, 25,36
X	SHIMIZU TADAMICHI ET AL: "High expression of macrophage migration inhibitory factor in human melanoma cells and its role in tumor cell growth and angiogenesis." BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 264, no. 3, 2 November 1999 (1999-11-02), pages 751-758, XP001008703 ISSN: 0006-291X abstract page 752, left-hand column, paragraphs 3,4 --- -/--	7-17, 20-23, 25,36-47

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/05933

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MITAMURA YOSHINORI ET AL: "Macrophage migration inhibitory factor levels in the vitreous of patients with proliferative vitreoretinopathy." AMERICAN JOURNAL OF OPHTHALMOLOGY, vol. 128, no. 6, October 1999 (1999-10), pages 763-765, XP001013411 ISSN: 0002-9394 abstract	42,43, 52,53
Y	ONODERA SHIN ET AL: "Macrophage migration inhibitory factor up-regulates expression of matrix metalloproteinases in synovial fibroblasts of rheumatoid arthritis." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 275, no. 1, 7 January 2000 (2000-01-07), pages 444-450, XP002174637 ISSN: 0021-9258 cited in the application the whole document	9,10
Y	WINTER G ET AL: "HUMANIZED ANTIBODIES" IMMUNOLOGY TODAY, ELSEVIER PUBLICATIONS, CAMBRIDGE, GB, vol. 14, no. 6, 1996; pages 243-246, XP001005438 ISSN: 0167-4919 the whole document	18,19
Y	HEIMAN A S ET AL: "New steroidal anti-inflammatory antedrugs bind to macrophage glucocorticoid receptors and inhibit nitric oxide generation - Physiology, pathophysiology and pharmacology" STEROIDS, BUTTERWORTH-HEINEMANN, STONEHAM, MA, US, vol. 63, no. 12, December 1998 (1998-12), pages 644-649, XP004151180 ISSN: 0039-128X the whole document	37-41

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims 1-25 and 36-53 of the present application relate partially to anti-MIF antibodies and fragment thereof (and (i) methods for preparing said antibodies, (ii) nucleic acids encoding said antibodies, (iii) therapeutic compositions comprising said antibodies and (iv) methods for treating various diseases using said antibodies),

wherein the antibodies are defined by reference to a desirable characteristic or property of said antibodies, namely that they bind to a "MIF-like protein or fragment thereof".

Since the meaning of the feature "MIF-like protein" is vague and unclear, the claims partially lack clarity (Article 6 PCT). It is noted that the feature "MIF-like protein" does not have a well-recognized meaning in the field and moreover, that this feature has not been precisely defined in the description of the present application.

The resulting lack of clarity is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to antibodies binding to MIF , MIF 1-3, or GIF (see description of the present application, pages 1, 2, 8 and Table I).

For the same reasons, claims 26-35 have only been partially searched, i.e. the unclear part relating to "MIF-like protein" has not been searched.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter. onal Application No
PCT/US 01/05933

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0162812 A	27-11-1985	AT 93869 T	15-09-1993
		DE 3587553 D	07-10-1993
		DK 229985 A	25-11-1985
		ES 543391 D	16-01-1987
		ES 8702950 A	01-04-1987
		ES 557071 D	16-02-1988
		ES 8801852 A	01-05-1988
		ES 557072 D	01-08-1987
		ES 8801850 A	01-05-1988
		IE 60450 B	13-07-1994
		JP 7070192 A	14-03-1995
		JP 7108915 B	22-11-1995
		JP 1952554 C	28-07-1995
		JP 6072158 B	14-09-1994
		JP 60248617 A	09-12-1985
		JP 2047584 C	25-04-1996
		JP 6217789 A	09-08-1994
		JP 7080915 B	30-08-1995
		AU 592753 B	25-01-1990
		AU 4281385 A	28-11-1985
		ZA 8503915 A	29-01-1986